ABSTRACT

BIOLOGY

JONES, BETTY RUTH B.S., Rust College, 1973

M.S., Atlanta University, 1975

Scanning Electron Microscopy and the Ultrastructural Localization of Alkaline Phosphatase and Acetylcholinesterase Activity in the Tegument of the Cysticercus of Hydatigera taeniaeformis

Advisor: Dr. Barnett F. Smith

Doctor of Philosophy degree conferred August 4, 1978

Thesis dated August, 1978

Scanning electron microscopy (SEM) and transmission electron microscopy (TEM) were used to study the surface topography and the ultrastructural localization of alkaline phosphatase and acetylcholinesterase activity in the tegument of the cysticercus of Hydatigera taeniaeformis. Examination of the scolex, strobila and bladder of the cysticercus of H. taeniaeformis by SEM revealed certain distinct features. The suckers were composed of large folds, depressions and ridges. At high magnifications microtriches were observed within the cavities and over the rims of the suckers. The rostellum consisted of a double crown of hooks which projected from large oval-shaped cavities. Indentations, folds and microtriches were observed on the surface of the scolex proper.

The tegument of the strobila was studded with bosses of uniform size which were revealed as microtriches at high magnifications. The pear-shaped bladder consisted of densely packed microtriches and lateral and terminal excretory pores.

Scanning electron microscopy of the eggs of H. taeniaeformis examined at high magnifications revealed the presence of scale-like patterns. At low magnifications the surface of the eggs appeared smooth. Eggs that were fractured showed a thick, inner surface layer of ridges. When the eggs were treated with different concentrations of ethylenediaminetetraacetic acid (EDTA), the surface became distorted and the emerging hooks of the embryo were revealed. Small, spherical bosses were observed on the surfaces of some eggs. Other eggs, possibly at an earlier stage of development, contained pit-like depressions.

Alkaline phosphatase activity in the larval tapeworm was studied by means of TEM. Beta-glycerophosphate was used as the substrate and 1 mM sodium fluoride (NaF) or 10 mM EDTA was used to inhibit alkaline phosphatase activity. The reaction product was localized within the microtriches of the distal cytoplasm and processes of perinuclear cells. Activity was demonstrated within circular and longitudinal muscle bundles and in calcareous corpuscles.

Acetylcholinesterase activity in the scolex of the cysticercus of H. taeniaeformis was also studied by means of TEM.

Acetylthiocholine iodide was used as the substrate and 10^{-4} M eserine was used to inhibit cholinesterase activity. The reaction product was localized within the microtriches of the distal cytoplasm, muscle bundles, fibrous tissue and cytoplasm and vesiculated bodies of the rostellar region. Acetylcholinesterase activity was also demonstrated within the nuclear membrane, cytoplasm, and membrane of nerve cells, flame cells, and hooks of the rostellum.

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CHAPTER I

INTRODUCTION

Cysticercosis is a condition caused by a tapeworm infection. The embryonated eggs or oncospheres inside gravid proglottids are passed in the feces of a definitive host. Man, pigs, dogs, cats, and other animals may ingest these eggs in contaminated food. The eggs are liberated in the small intestine and make their way through the gut, wall of the blood vessels, and are carried to all parts of the body. In various organs, the larval worms leave the blood and develop into the cysticercus stage (Noble and Noble, 1971).

Cysticercus fasciolaris is the larval form of the adult cat tapeworm, Hydatigera taeniaeformis (= Taenia taeniaeformis). It is of increasing interest because of the considerable pathological conditions produced in a rat or mouse host in which it is normally found. The larvae encapsulate and develop on the liver of rats and mice. A serious cancer-like growth apparently may arise from this encapsulation (Noble and Noble, 1971).

To our knowledge there have not been any studies reported on SEM of the eggs of C. fasciolaris. An electron scan of trematode eggs of Schistosoma mansoni was reported by (Race et al., 1971). A few studies have been reported on the surface topography of the eggs of nematodes (Allison

et al., 1973). These observations were made on Ascaris lumbricoides, A. suum, Toxocara canis and Porrocaecum sulcatum. Recently, Ubelaker and Allison (1976) also used SEM to study the surface topography of the eggs of A. lumbricoides, A. suum, T. canis and T. mystax. Therefore, one of the objectives of the present study was to use methods of SEM to study the surface morphology of the eggs H. taeniaeformis.

Only a few studies could be found on SEM of the larvae of cestodes. Ubelaker et al., (1973) described the surface topography of Hymenolepis diminuta. Microtrichial polymorphism among hymenolepid tapeworms as seen by SEM was reported by Berger and Mettrick, 1971. Other studies have been described in trematodes and nematodes. Race et al. (1971) described the SEM of the cercariae of S. mansoni. The integumental surface of S. mansoni was reported by Miller, Tulloch and Kuntz, 1972. Recently Tulloch et al. (1977) described the fine external morphology of S. mattheei. Scanning electron microscopy of the nematodes, Sulcascaris sulcatum and Cotylaspi insignis, has been reported by Allison, Ubelaker, Webster and Riddle, 1972. In the present study SEM was used to study the surface topography of the cysticercus of H. taeniaeformis.

Localization of alkaline phosphatase activity in cestodes has been extensively investigated through light microscopy. Rogers (1947) demonstrated alkaline phosphatase activity in

the tegument of Moniezia expansa. Yamao (1952a,b) reported on alkaline phosphatase activity in several adult cestodes, such as Anoplocephala perfoliata, A. magna, M. benedeni, M. expansa and T. taeniaeformis, and also reported on the larval forms, Cysticercus bovis, Echinococcus cysticus fertilis and C. fasciolaris. Erasmus (1975a,b) reported on the adults, M. expansa, Echinococcus granulosus, and the larvae, Cysticercus tenuicollis; Kilejian et al. (1961) on E. granulosus; Bogitsh (1963,1967) on Hymenolepis microstoma and cysticercoids of H. diminuta and H. microstoma; Waitz (1963) on the cysticercus and adult of H. taeniaeformis; Waitz and Schardein (1964) on H. diminuta, H. nana, H. taeniaeformis and Dipylidium caninum; Lee and Tatchell (1964) on A. perfoliata, and Arme (1966) on Ligula intestinalis. A few investigators have reported on the ultrastructural localization of alkaline phosphatase activity in cestodes (Rothman, 1966, H. citelli; Lumsden, 1968,1975, H. diminuta and Dike and Read, 1971, H. diminuta). However, the work of these investigators was limited to the microthrix brush border. The present study was undertaken to investigate the ultrastructural localization of alkaline phosphatase activity in the tegument and subtegumental regions of the cysticercus of H. taeniaeformis.

Extensive review of the literature has revealed that the work of Morseth (1967) was probably the first report on the ultrastructure of the nervous system of cestodes. Among the

numerous studies on the ultrastructure of these forms, none have dealt with the localization of acetylcholinesterase. Rothman (1966) localized esterases in the tegument of H. citelli.

Previous histochemical studies on the nervous system of cestodes, have in fact, been largely restricted to the use of light microscopy with simple whole preparations and sectioned materials. Investigators which have used this approach include Hart (1967) with tetrathyridium of Mesocestoides corti; Ramisz (1967), Delepis undula and Choanotaenia unicoronata; Wilson and Schiller (1969), Hymenolepis sp.; Shield (1969), Dipylidium caninum, E. granulosus and H. taeniaeformis; and LeFlore and Smith (1976), C. fasciolaris.

Cholinesterases have also been localized histochemically in the nervous system of a number of other cestodes (H. diminuta, H. nana, H. taeniaeformis and D. caninum, Lee, Rothman and Senturia, 1963); Schardein and Waitz, (1965); Wilson (1965); and Douglas (1966); A. perfoliata, Lee and Tatchell (1964); Ligula intestinalis, Arme (1966); in cysticercoids of H. diminuta and H. microstoma, Bogitsh (1967), and in embryos of H. diminuta, Rybicka (1967).

In addition some biochemical reports have revealed the presence of this activity in H. diminuta and in hydatid scolices and brood capsules of E. granulosus (Graft and Read 1967); and Schwabe et al. 1961). The enzymes in these studies however, were demonstrated from homogenates and their sites

of activity were not indicated.

The present investigation, therefore, was designed to study the localization of acetylcholinesterase activity in the scolex of the cysticercus of H. taeniaeformis with the expectation that a more intricate knowledge of the sites of activity of the enzyme may be obtained.

CHAPTER II

REVIEW OF LITERATURE

Rees (1951) described the anatomy of C. fasciolaris from the liver of Rattus norvegicus. Detailed studies were made of the musculature, excretory and nervous systems, using whole mounts and serial sections. Emphasis was also placed on the external features of C. fasciolaris. The body of the larva was elongated, varying in length according to age. The smallest specimen measured was 4.0 mm in length and the largest 150.0 mm. The body was segmented. At the posterior extremity was a pear-shaped bladder that measured from 1.0 to 5.0 mm in diameter, according to the size of the worm. The scolex measured 1.76 mm in breadth in one of the largest specimens. Each of the four acetabula measured 0.35 mm in diameter. The rostellum was armed with a double crown of 36 hooks, though the number varies from 26-52 in various species. The anterior hooks were the largest, measuring 0.38-0.41 mm in length and those of the posterior row, 0.25-0.27 mm. The hooks of both rows had a curved blade and a slightly bifid guard.

An elemental, amino acid and infrared spectrophotomeric analysis of the hooks of H. taeniaeformis was studied by (Dvorak, 1969). The early development of the rostellum of C. fasciolaris and the chemical nature of the hooks were reported by (Crusz, 1947). A critical study of the taxonomy

of some cestodes, including H. taeniaeformis, was made by (Esch and Self, 1965). It was concluded that the size of long and short rostellar hooks provided the most reliable criteria for separating species. Voge (1963) made observations on the structure and function of cysticerci of T. solium and T. saginata. In spite of minor apparent differences, the structures of the two species was very similar and their specific differentiation could not be guaranteed when the scolex was not available.

Hutchinson (1958) studied the growth of the larval stages of H. taeniaeformis. It was observed that the larvae developed on the 30th day of infection. On the 42nd day a strobilocercus formed. The youngest strobilocercus, which was 60 days old, was recovered from a rat and was capable of infecting a cat. There was rapid growth in the first six weeks, after which the growth rate slowed down. The complete growth period was 22 weeks.

Lewert and Lee (1956) used histochemical methods to determine the state of acellular proteins in the liver of a host infected with the larvae of H. taeniaeformis. It was found that during the initial stages of the infection and early growth of the larvae in the host, the acellular glycoproteins of the liver were altered and hepatic cells surrounding the larvae were depleted of glycogen. At the formation of capsules around the larvae, the adjacent liver cells were normal with respect to polysaccharides containing protein and glycogen.

Race et al. (1971) studied the eggs of S. mansoni by SEM. The eggs showed a prominent spine, a wrinkled or folded outer layer and projections from the surface. High magnifications of the posterior lateral surface near the spine revealed that the outer layer of the shell contained many separate depressions. SEM of the eggs of A. lumbricoides, A. suum, T. canis and T. mystax were studied by (Ubelaker and Allison, 1976). All species exhibited pronounced surface ridges. Ridges formed distinctive patterns in T. canis and T. mystax. In Ascaris species, the ridges were similar except that they were more pronounced in A. suum. Operculum-like structures were observed only in Ascaris. Correlation of the data of SEM with that of TEM suggested that the surface ridges seen in AScaris eggs were formed by the chitinous layer of the shell.

Specian et al. (1973) reported on the preparation of amyl acetate and acetone labile eggs of nematodes for SEM. After preparation with glycerol, the surfaces of the eggs from T. mystax, T. canis and A. suum appeared as elaborate reticular networks. These networks divided the surfaces into irregular ridges and depressions. The depressions were smaller and more numerous in T. mystax and larger and less numerous in A. suum. The eggs of Toxocara species did not appear to possess an operculum-like region. Preparation of A. suum eggs by the critical point drying method which employed either acetone or amyl acetate rendered the surfaces smooth by removing or distorting the characteristic ridges.

Ubelaker and Allison (1972) studied the denticles of the eggs of A. lumbricoides and A. suum by SEM. The denticles of A. suum were regular in distribution and conoid in shape. Each denticle was cuspid. No multiple cusps were observed. Denticles of A. lumbricoides were regular in distribution and were noticeably different in size. Cusps were typically single and occasionally appeared flattered on the surface. The cusp surface was rarely conoid but appeared irregular in the outline with several small protuberances on the surface. Both species revealed an elaborate reticulin network which divided the surface into irregular depressions. At one pole of each ovoid egg the surface layer was modified by the formation of a large rounded depression. The significance of this polar modification was not understood, but it was believed that it represented a region through which the larval form emerged from the egg.

Belton (1977) did a freeze-fracture study of the tegument of the larva T. crassiceps. This freeze-fracture study correlated with the fine structure of the parasite described by (Baron, 1968). SEM of H. diminuta was reported by (Ubelaker, Allison and Specian, 1973). The surface of H. diminuta showed that that dense populations of microtriches occurred on the rostellum, suckers and scolex.

Berger and Mettrick (1971) studied microtrichial polymorphism among Hymenolepid tapeworms as seen by SEM. Three species of Hymenolepis revealed polymorphic microtriches. In H. diminuta, ontogenetic allometric growth resulted in mature

and early gravid segments having two distinct populations of posteriorly-oriented microtriches. One population was short and tubular with conical tips. Another population was long and flattened with spatulate tips. The microtriches of H. nana were polymorphic and oriented at right angles to the tegument. In H. microstoma, microtrichial dimorphism was apparent in the neck and immature strobila region. The large microtriches in the neck of this species pointed in many directions, but most assumed a posterior orientation as the segments matured. All three species exhibited a decreased microtrichial density down the length of the strobila and morphological changes in the tegumental surface of gravid segments. It was suggested that the larger microtriches may play a role in site-selection and intra-luminal migrations of these worms. Relatively small increases in surface tegumental areas due to microtriches suggest that they do play an important role in nutrient absorption.

Boyce (1976) described a new organ in the surface ultrastructure of Eubothrium salvelini. These organs designated as 'tumuli' were found on at least two other representatives of the order pseudophyllidea. SEM of the integument of S. mansoni was reported by (Tulloch et al. 1977). Marked differences on the surface were observed as well as different regions on the same parasite. The male of S. mattheei possessed noticeable bosses that were devoid of spines or

integumental thickenings. The female was characterized by a smooth integument with minor elevations or remnant bosses.

Kuntz et al. (1976) studied the integumental surfaces of S. haematobium by SEM. The orientation of bosses consisted of blunt, irregularly shaped, low elevations in the male. The surfaces of the female were slightly rough. SEM of the integumental surfaces of S. mansoni was reported by (Miller et al. 1972). Many spines of variable size covered the inner surface of the oral sucker of the male and extended to the pharyngeal opening. Fewer and smaller globular spines covered the inner surface of the ventral sucker. Bosses of spines were on the greater part of the body surface but not in the gynecophoral canal. The posterior end of the male was devoid of bosses but spines were freely distributed over the surface. The female was free of bosses, but spines covered the posterior part of the body.

Race et al. (1971) studied cercariae and adults of S. mansoni by SEM. The integument of the male revealed a rough external surface and that of the female was smooth. The cercariae possessed a smooth surface. Allison et al. (1973) reported on the SEM of P. sulcatum, a nematode from the sea turtle. The anterior region of the subventral lip consisted of a double papilla and protuberance at the margin. The auricle consisted of transverse grooves and longitudinal ridges. Conical denticles extended laterally along the lip margins. SEM of the nematodes S. sulcatum and C. insignis

was reported by (Allison et al. 1972). Both species possessed papillae and denticles. C. insignis consisted of a series of alveoli.

Martin (1973) made an electron scan of the nematomorph cuticle of male and female horse-hair worms.

There have been no studies reported on the SEM of the bladder of cysticerci, a structure that has been described by Šlais (1966) as a transitory embryonic organ. Its morphology is related to the mode of development and localization of the parasite in its intermediate host. According to this investigator, the bladder makes possible the adaptation of the larva to unusual conditions during its development. Several investigators have suggested that the bladder of related forms was a specialized structure which played an important role in nutrition and metabolism as well as a storage reservoir for growing strobila rather than functioning exclusively as an excretory structure (Šlais, 1966; Taylor, McCabe and Longmuir, 1966).

Nieland and Weinbach (1968) described the ultrastructure of the bladder of C. fasciolaris. Projections were observed on the external surface of the tegument. These projections were called microtriches. Cytoplasmic bridges provided continuity between tegumental cytoplasm and subtegumental cells. The tegumental cytoplasm was packed with small vesicles and mitochondria. Rough endoplasmic reticulum, free ribosomes and duct-like profiles were also observed, as well

as nucleoli and fat droplets which were surrounded multiple membranes.

The alkaline phosphatases belong to the group of phosphomonoesterases. These are nonspecific enzymes that hydrolyze virtually all monoesters of phosphoric acid with the liberation of orthophosphate. These enzymes are active on the alkaline side of neutrality, especially at pH 9.0 and above. The ultrastructural localization of phosphatase activities is based largely on the application of histochemical procedures involving the precipitation of metal salt procedures which were originally developed for light microscopy by Gomori (1939) and Takamatsu (1939) independently. These methods are based upon the theory that phosphate ions, liberated by enzymatic hydrolysis of various organic phosphates serving as substrates, will be trapped at the site of formation by metal cations present in the incubation medium to form highly insoluble precipitates. The precipitates of lead or calcium are most frequently used as trapping ions and are transformed into blackish, highly insoluble reaction products (Hayat, 1973).

The procedures for the localization of alkaline phosphatase reaction product are generally based on either the direct or indirect (two-step) precipitation of a heavy metal such as calcium, cobalt, silver, lead or cadmium. In the original method for light microscopy, calcium is used at alkaline pH to trap the phosphate liberated from the substrate

by enzymatic hydrolysis (Gomori, 1952). The resulting primary reaction product, calcium phosphate, is converted in a second step into cobalt phosphate and finally into cobalt sulfide. Calcium phosphate has only moderate opacity to electrons, but it can be increased according to Brandes et al. (1956) by converting the precipitate into a silver salt.

The same result can be achieved by converting calcium phosphate into a lead salt (Molnar, 1952; deThe, 1965). This is carried out by treating the incubated sections with 2% lead nitrate for a few minutes (Wetzel, Spicer and Horn, 1967). In addition to the increased opacity, one advantage of lead salts as a final reaction product is that they are relatively resistant to post-fixation in osmium tetroxide (Reale and Luciano, 1964).

The physiological significance of phosphomonoesterases was reviewed by Moog (1945). The alkaline phosphomonoesterases appear to be salt-soluble and the acid phosphomonoesterases appear as water-soluble substances. Both consist of a metallic-like enzyme and a protein carrier. Neither depends on sulfhydryl groups for activity, but the alkaline phosphatase seems to require amine and phenolic hydroxyl groups and is not affected by strong oxidizing agents. The phosphatases were found also to catalyse hydrolyses, not syntheses.

Rogers (1947) studied the histological distribution of

alkaline phosphatase activity in A. lumbricoides from the intestine of the pig and M. expansa from the small intestine of the sheep. The enzyme was present in mature segments of the cestode in considerable amounts. Spherical bodies in the intestinal cells of the nematode, A. lumbricoides, showed a phosphatase reaction. No other tissues of the parasite had a positive reaction. The parasites examined showed the greatest concentration of alkaline phosphatase in those regions involved with the absorption of carbohydrate. It was concluded that these observations were in accord with the hypothesis that phosphorylation mechanisms are involved in the absorption of glucose in the intestine and kidney.

Yamoa (1952a) using methods for light microscopy, studied the histochemical distribution of acid and alkaline phosphatase activity in the adult cestodes, A. perfoliata from equine caecum and small intestine, A. magna from equine small intestine, M. expansa from the small intestine of sheep, T. taeniaeformis from the small intestine of cats. According to this investigator, generally both alkaline and acid phosphatases were in the cuticle where nutritional absorption is assumed to be carried out, although some differences are found arising from differences in species and aging of proglottids. A positive alkaline phosphatase reaction was strongest in the basal membrane, followed by the subcuticular cells, while no reaction was seen in the cuticular layer.

An equally strong positive acid phosphatase reaction was

observed in the cuticular layer and in the subcuticular cells. Outside the cuticular layers, a positive alkaline phosphatase reaction was found in the epithelia of the excretory ducts. An exception was seen in T. taeniaeformis in which neither phosphatases could be detected. However, comparison of the two phosphatases showed that the alkaline phosphatase was distributed more widely and had a stronger positive reaction than the acid phosphatase.

Phosphatase distribution was also observed by Yamoa (1952b) in the larvae of cestodes. The following larvae were examined: C. bovis from bovine muscles, E. cysticus fertilis from hog liver and C. fasciolaris from rat liver. The cuticular layer of the cyst wall where larvae were attached showed a characteristic structure almost identical with the villus of the small intestine in mammals. The reaction of acid and alkaline phosphatase activity was strongly positive in this area of the cyst wall, which probably means that absorption of nutritious matter from the host was being carried out at this point. On the internal surface of the larvae, acid phosphatase alone was positive in C. bovis, while a positive alkaline phosphatase was also found in E. cysticus fertilis. Alkaline phosphatase activity was also present in the cuticle of the excretory duct of these species. The distribution of both phosphatases in the body surface cuticular layer of C. fasciolaris was identical with that in the adult worm. Neither phosphatase could be detected

in the cuticle of the larval excretory duct.

Erasmus (1957a) studied the phosphatase systems in the adult and cysticercus stages of T. pisiformis. Histochemical tests failed to demonstrate the sites of enzyme activity in the Cysticercus. In the adult, the acid phosphatase was confined to the cuticle, subcuticular cells and membranes bounding the ovary and vitelline tubules. The histochemical distribution was uneven along the length of the worm, both acid and alkaline phosphatase being predominant in the region of the mature proglottids. The scolex was negative to both tests. Biochemical tests demonstrated distinct acid and alkaline phosphatase activity in the Cysticercus and adult stages. In the Cysticercus the acid enzyme was predominant and in the adult, the alkaline enzyme was predominant.

The phosphatase systems in the larva cestode, Cysticercus tenuicollis and the adult, M. expansa were also investigated by Erasmus (1957b). Attempts to locate the enzymes by normal histochemical methods were unsuccessful in C. tenuicollis. In M. expansa, acid and alkaline phosphatase occurred in the cuticle. Alkaline phosphatase was present in the tunicae enclosing the ovary, vitelline glands and testes. This enzyme was also present in the interproglottid glands, in the walls of the developing uterus and in the subcuticular cells. Tests for all types of phosphatase activity showed that cuticular activity in M. expansa originated in the wall of an extensive branched system of channels. Incubation experiments with

living M. expansa showed that substrates external to the body were capable of being broken down. Similar tests with C. tenuicollis showed that at least some of the alkaline phosphatase recorded by biochemical tests was located in the cuticle.

Kilejian et al. (1961) studied the distribution of acid and alkaline phosphatase activity in E. granulosus. Strong alkaline phosphatase activity was present in the cuticle of the adult worm, but no acid phosphatase activity could be demonstrated. The larval scolices contained minute amounts of both acid and alkaline phosphatase, and the germinal membranes only small amounts of the acid enzyme. The laminated membrane gave a negative reaction for both enzymes.

Bogitsh (1963) demonstrated the distribution of acid and alkaline phosphatase in H. microstoma. The distribution of the phosphatases was primarily cuticular. In addition, alkaline phosphatase activity was found in the subcuticula and in the walls of the excretory ducts. Acid phosphatase was confined to the cuticula; the greatest indication of activity of this enzyme was seen in the outermost portions of the cuticula. This same investigator in 1967 studied the histochemical localization of phosphatase activity in H. diminuta and H. microstoma. The intermediate cell layers of cysts surrounding the larvae of H. diminuta and H. microstoma showed acid phosphatase activity. This activity was optimal at pH 5.0. Another phosphatase was associated with the larval

teguments. This phosphatase had a pH range of 6.0-9.0 and was believed to be associated with lysosomes and encystation.

Waitz (1963) found acid and alkaline phosphatase activity in the larva and adult of H. taeniaeformis. Acid phosphatase activity was found in highest concentrations in the cuticle; the subcuticular cells showed faint activity, while the parenchyma was negative or only slightly positive. Alkaline phosphatase activity was intensest in the cuticle and subcuticular cells; a faint reaction was around the parenchymal musculature. Waitz and Schardein (1964) found acid and alkaline phosphatase activity in H. nana, H. diminuta, H. taeniaeformis and D. caninum. The cuticle of all four species contained high acid phosphatase activity. The subcuticular cells generally had less of enzyme activity and usually showed a weakly staining granulation, but such staining was most intense in H. nana. All other structures were negative. The cuticle of all four species examined showed high alkaline phosphatase activity. The subcuticular cells stained uniformly intense in H. diminuta and H. taeniaeformis and somewhat less intensely in H. nana and D. caninum. The parenchyma and its included structures were negative in fixed frozen sections of all species, but in cryostat sections of both species of Hymenolepis and H. taeniaeformis, slight activity was found in the parenchyma and parenchymal muscles.

Lee and Tatchell (1964) found considerable amounts of alkaline phosphatase activity in A. perfoliata. Activity was

found in the parenchyma of the free margins of the proglottids and lesser quantities were found in the general parenchyma. Enzyme activity was apparent in the microvilli of the anterior face of the proglottids. Arme (1966) demonstrated alkaline and acid phosphatase activity in the cestode Ligula intestinalis. Phosphatases were found mainly in the cuticle and subcuticular cells. These enzymes were demonstrated in both the plerocercoid and adult stages; however, alkaline phosphatase was predominant in the adult.

Only a few investigators have reported on the ultrastructural localization of alkaline phosphatase activity in cestodes. These studies have been limited to the microthrix brush border. Rothman (1966), using ultrastructural methods demonstrated alkaline phosphatase activity on the outer membrane of the proximal microtriches in H. citelli. The precipitate accumulated on the surface of the proximal microtriches in large amounts. Activity on the distal microtriches and in the mitochondria was questionable. The scolex reacted differently from the strobila region. No alkaline phosphatase activity was seen in the cuticle of the scolex.

Lumsden et al. (1968) in studying the absorptive surfaces of H. diminuta demonstrated the hydrolysis of a variety of phosphate esters, including mono- and triphosphate nucleosides of adenine, guanine, cytosine hexose and glycerophosphate. Activity was localized largely at the external surface of the plasmalemma bordering the free surface of the strobila integument. A means by which surface phosphatases could

facilitate absorption from the parasites' microenvironment was suggested. Lumsden (1975) in a review paper on the surface ultrastructure and cytochemistry of parasitic helminths indicated that surface activity served an important digestive function, converting otherwise untransportable compounds into components which can cross the membrane. Dike and Read (1971) demonstrated tegumentary phosphohydrolases of H. diminuta using fructose phosphate and para-nitrophenyl phosphate as substrates. Ultrastructural localization of enzyme activity showed both substrates to be hydrolyzed at the brush border of the tegument.

Many species of both trematodes and cestodes contain large numbers of curious concretions termed calcareous corpuscles. These are especially noticeable in larval forms and the numbers present vary widely even in the same species. Calcareous corpuscles consist of an organic base together with inorganic material. The inorganic material has been reported to contain DNA, RNA, proteins and alkaline phosphatase. The inorganic materials consist mainly of calcium, magnesium, phosphorus and carbon dioxide. Although the function of calcareous corpuscles in cestodes is not known, it has been suggested that in H. taeniaeformis the function of calcareous corpuscles could be to buffer an aerobically produced acid (Smyth, 1969).

There have been no published reports on the localization of alkaline phosphatase activity in calcareous corpuscles in which histochemical methods for electron microscopy were

utilized. Chowdhury and Ray (1962) used light microscopic methods to study the nature and structure of calcareous corpuscles in T. saginata. An alkaline phosphatase reaction in the form of a black deposit of cobalt sulfide was obtained in calcareous corpuscles. The corpuscles were found throughout the parenchyma, even in the region of the scolex. Structurally, each corpuscle had a concentric appearance. Scott et al. (1962) studied the mineralogical composition of calcareous corpuscles in T. taeniaeformis and found calcium, phosphorus and carbon dioxide to be the major components.

von Brand et al. (1960) made observations on the function, composition and structure of calcareous corpuscles in the larva and adult of T. taeniaeformis. Of increasing interest was the observation that the larval form contained much more corpuscle material than did the adult. von Brand et al. (1965) also studied the mineralogical composition of calcareous corpuscles in Cysticercus cellulosae, larval Spirometra mansoides, T. saginata and Diphyllobothrium latum. Nieland and Weinbach (1968) studied the ultrastructure of calcareous corpuscle formation in T. taeniaeformis. The corpuscle formed in a cytoplasmic cavity and the corpuscle-forming cell appeared to extend itself in the production of one corpuscle. Two corpuscular components were recognized, a homogeneous matrix that corresponded to organic moities and a granular substance that represented inorganic materials.

Acetylcholinesterase is an enzyme that belongs to a larger group of enzymes known as esterases. Esterases are a

heterogeneous group of enzymes widely distributed in animal and plant tissues. They hydrolyze the carboxylic acid esters of alcohols, phenols and naphthols. The esterases are divided into five groups based on their reactions with different substrates and their sensitivities to inhibitors. These five groups are as follows: carboxylesterases, arylesterases, acetylesterases, acetylcholinesterases or true cholinesterases, and cholinesterases or pseudocholinesterases (Hayat, 1974).

Acetylcholinesterases hydrolyze choline esters at a higher rate than aliphatic or aromatic esters. These enzymes are inhibited by low concentrations of physostigmine (eserine) or organophosphorus compounds. Physostigmine or eserine is one of the best known inhibitors of cholinesterase activity. It is an alkaloid containing a carbamino group. It is obtained from calabar beans, the seeds of a West African vine, Physostigmina venosum Balfouri. Eserine is an extremely potent anticholinesterase and has two main uses as an experimental tool. In bioassay, it will prevent the cholinesterase of a test organ from hydrolyzing any acetylcholine. In biochemical or histochemical studies, it is necessary to distinguish reactions which may be due to cholinesterases from those due to other eserine-resistant esterases. For most vertebrate species, a concentration of 10^{-4} M or 10^{-5} M eserine is more than adequate ot inhibit all cholinesterase activity in vitro. If a reaction still occurs despite the presence of 10^{-4} M or 10^{-5} M of eserine it is unlikely to be due to a cholinesterase. Clinically, eserine is applied topically in the

treatment of glaucoma but in most instances where an anticholinesterase is required for medical use, the more stable compound, neostigmine, is preferred (Silver, 1974).

According to Silver (1974) there is little difficulty in assigning a function to cholinesterase in nerve tissue, but in many cases, specific acetylcholinesterase as well as nonspecific cholinesterase activity has been found in nonnervous tissue. For example, both acetylcholinesterase and cholinesterase activity have been found in erythrocytes and platelets. It is thought that in this case acetylcholinesterase may be involved in erythropoiesis. Both enzymes have been found in vascular tissue and are assumed to play a role in the maintenance of blood. Acetylcholinesterase activity has also been found in the placenta of certain organisms, and it is believed that this enzyme from the placenta may provide the newborn animal with red blood cell acetylcholinesterase. Samuels, Moller and Fischer (1968) suggested that acetylcholinesterase may have a metabolic function in some tissues.

The thiocholine copper ferrocyanide method for demonstrating acetylcholinesterases at the electron microscopy level was introduced by Karnovsky (1964) and Roots (1964). This method employs thiocholine esters as substrates and the capture reaction is the reduction of ferricyanide ions in the medium by free thiocholine. In the presence of copper the reaction product is the colored, electron opaque, copper ferrocyanide. In the absence of copper ions no reaction product can be demonstrated. The thiocholine copper

ferrocyanide technique has the merit of using specific substrates, acetylthiocholine iodide or butyrylthiocholine iodide (Pearse, 1972).

As to our knowledge, there have been no studies on the ultrastructural localization of acetylcholinesterase activity in the scolex of the cysticercus of H. taeniaeformis. Simple whole-mount preparations and sectioned materials of cestodes for light microscopy have been studied extensively. Hart (1967) studied the nervous system of intact tetrathyridia of Mesocestoides corti using the Gerebtzoff modification of the Koelle and Friendenwald cholinesterase technique. The scolex revealed a black cap appearance. This black cap region contained a very dense array of cells and fibers. The broad cholinesterase reaction of this region suggested the presence of a more dense array of nervous elements in an organism which undergoes asexual division of presumptive nervous elements for duplication of the nervous system and suckers. Eserine at a concentration of 10^{-4} M and diisopropylphosphorofluoridate (DFP) at a concentration of 10^{-6} M were used to inhibit the cholinesterase. Eserine completely inhibited any visible reaction with both substrates, acetylthiocholine and butyrylthiocholine iodide. Diisopropylphosphorofluoridate inhibited the reaction with butyrylthiocholine and sharply reduced the reaction with the acetylthiocholine iodide.

Ramisz (1967) described the nervous system in the cestodes Dilepis undula and Choanotaenia unicoronata. The

arrangement of longitudinal and transverse nerves in D. undula was a ladder type and in the uterine segment of C. unicoronata it gradually turned into a nerve net. The innervations of the genital systems in these tapeworms was also described. The function of the genital system in these tapeworms was found to be regulated by acetylcholinesterase. Wilson and Schiller (1967) demonstrated the distribution of acetylcholinesterase activity in whole-mounts of H. diminuta and H. nana. It was suggested that acetylcholinesterase activity in these organisms could be correlated with a neuromuscular function or with the presence of neurosecretory glands and chemoreceptors.

Shield (1969) studied cholinesterases in D. caninum, E. granulosus, and H. taeniaeformis to demonstrate the nervous system. Some cholinesterase inhibitors were used to characterize the enzyme. Eserine completely inhibited cholinesterase activity. Para-chloromercuri-benzoate (PCMB) reduced enzyme activity. Mipafox (N,N-di-isopropylphosphoropdiamedic fluoride) and tetra-mono-iso-propylpyrophosphorotetramide (iso-OMPA) had little effect, while DFP and diethyl-p-nitrophenyl phosphate (E-600) were effective only at relatively high concentrations. It was concluded by these investigators that there was an acetylcholinesterase in the species examined, and that butyrylcholinesterase was not present in E. granulosus and that there were no nonspecific esterases.

LeFlore and Smith (1976) used the acetylthiocholine iodide method to demonstrate the nervous system of C. fasciolaris. The nervous system consisted of ten longitudinal nerve cords with transverse commissures in the strobila, and of ganglia and a complex system of commissures in the scolex. The longitudinal trunks terminated in the bladder as a nerve net which ramified throughout the tegument. Cholinesterases were inhibited by 10^{-3} M and 10^{-4} M eserine.

Lee et al. (1963) studied the distribution of esterases in H. taeniaeformis and in several species of Hymenolepis. Acetylcholinesterase activity was found in the parenchyma. Eserine at a concentration of 10^{-4} M inhibited cholinesterase activity but did not inhibit the uptake of glucose. These workers suggested that since tapeworms have no alimentary tract and since nutrient materials have to pass across the cuticle, it is possible that the acetylcholinesterase found in the cuticle of cestodes functions in the transport of sodium across the cuticle. Lee and Tatchell (1964) used the acetylthiocholine iodide method and demonstrated cholinesterase activity in the excretory and nervous system of A. perfoliata. In the excretory system, the reaction was located in the walls of excretory vessels. The nervous system produced an intense reaction in the longitudinal trunks, the ramifying nerve fibers and muscles. It was suggested that in general cholinesterases were concerned with nerve control and general metabolic functions. Schardein and Waitz (1965) studied

esterases in the cuticle and nerve cords of H. diminuta, H. nana, D. caninum and H. taeniaeformis. These enzymes were primarily localized in the cuticle and nervous system, but with quantitative and qualitative differences from species to species. Inhibition studies suggested that the enzyme hydrolysing both acetate and thiocholine substrates was an eserine, fluoride and organic phosphate-sensitive choline esterase. Partial inhibition by a strong ammonium base and hydrolysis of two choline esters indicated that both acetycholinesterase and pseudocholinesterase were involved.

Wilson (1965) demonstrated acetylcholinesterase activity in the scolex of H. diminuta and H. nana. He reported that the extensive innervation of the rostellum of H. diminuta, as compared with that of H. nana, indicated that in the former the rostellum may be primarily sensory in function, rather than being a remnant of a muscular organ of attachment, as has been commonly assumed. Arme (1966) demonstrated esterase activity in the subcuticular cells of the plerocercoid and adult of L. intestinalis. Differentiation of esterases by various inhibitors revealed the existence of three types of esterases- a, b, and c esterases. The a-esterases were not inhibited by E-600 but by PCMB. A positive reaction was found in the main longitudinal nerve cords. The parenchyma of the adult exhibited a positive reaction and a little activity was found in the parenchyma of the larva. It was mentioned by this investigator that although helminth

esterases may not share the exact properties of those found in mammals, utilization of the above schemes has provided a basis for classification. It was indicated that the apparent association of esterases with lipids and excretory canals could indicate that the enzymes were concerned with the excretion of lipid which accumulates as a metabolic by-product formed during the rapid three-day maturation of the adult in the final host.

Bogitsh (1967) found a specific cholinesterase in the tegument and the nerves of the cysticercoids of H. diminuta and H. microstoma. Activity was inhibited by 10^{-4} M eserine. He concluded that the presence of cholinesterase activity may be indicative of membrane transport. According to Rothman (1966) this function may be lost by the scolex tegument in adult worms.

Graff and Read (1967) demonstrated specific acetylcholinesterase activity biochemically in H. diminuta. Acetylcholinesterase activity was inhibited by eserine but not by DFP. Schwabe et al. (1961) found a specific acetylcholinesterase present in homogenates of hydatid scolicies and brood capsules of E. granulosus. Enzyme activity was partially inhibited by eserine. On the basis of these observations it was suggested that the enzyme was involved in permeability control and osmoregulation. Rothman (1966) demonstrated ultrastructurally cholinesterase activity within the distal microtriches of H. diminuta. Activity was inhibited with eserine using the thioacetate substrate.

CHAPTER III

MATERIALS AND METHODS

Preparation of Cysticerci for SEM

Twenty day-old male Albino, Sprague-Dawley and Long-Evans rats were infected intragastrally with the onchospheres of H. taeniaeformis collected from gravid proglottids of infected cats. The proglottids were macerated with a mortar and pestle in order to liberate the onchospheres. This macerated material was administered to rats which were later sacrificed at intervals varying from 6 to 10 weeks after infection. The cysts were dissected intact from infected livers and washed in cold 0.85% physiological saline. The strobilocerci were liberated by gently rupturing the cyst walls with fine scissors. The scolex and bladder of the strobilocercus were fixed in 4% glutaraldehyde buffered with 0.1 M sodium cacodylate at pH 7.4, rinsed in buffer, post-fixed in osmium tetroxide, and dehydrated in a graded series of alcohols, modified after Sabatina et al. (1963).

For the electron scans the specimens were placed in amyl acetate and then into a drying apparatus (Paar Instruments, Moline II) for critical point drying with carbon dioxide. After drying, the specimens were coated with carbon and gold-palladium and examined in a Cwikscan 100-4 scanning electron microscope. The specimens were photographed using Polaroid P/N film at desirable magnifications.

Preparation of Eggs for SEM

Gravid proglottids of H. taeniaeformis collected from infected cats were macerated with a mortar and pestle. The eggs were then fixed for 2 h in 4% glutaraldehyde buffered with 0.1 M sodium cacodylate at a pH of 7.4. The solution containing the fixed cells was drawn into a 13 mm diameter Flutronic filter (0.45 pore size, Selas Dresher, Pa.) in a Swinnex holder (Millipore, Bedford, Ma.) and then extensively washed with cacodylate buffer containing 0.01 M or 0.02 M EDTA. The EDTA served to solubilize and to allow removal of some of the polysaccharide (Chet and Kisler, 1973).

The eggs were dehydrated in a series of 30, 50, 70, 85 and 95% ethanol for 10 min and three changes of 100% ethanol for 10 min each. Following dehydration, the eggs were placed in 100% amyl acetate for 2 h, critical point dried with liquid carbon dioxide in a drying apparatus, coated with carbon and gold-palladium and exmained in a Cwikscan 100-4 scanning electron microscope. Specimens were photographed using 55 P/N film at desirable magnifications.

Preparation of Cysticerci for TEM

Ultrastructural Localization of Alkaline Phosphatase Activity

Three-month-old infections of the cysticercus of H. taeniaeformis were dissected from cysts present on the liver of Long-Evans rats. The worms were relaxed in 0.85% physiological saline

and the strobila region of the tegument was cut into 2 mm pieces. Tissues were rinsed in buffer and frozen in a cryostat (Ames Model II).

Frozen tissues were sliced with a single edge razor blade about 50 mμ in diameter and incubated according to the lead precipitation method of Gomori (1952). Incubation was carried out in the following mixture: 100 mg sodium beta-glycerophosphate, 8.5 ml distilled water, 10 ml 0.2 M tris-maleate buffer at pH 10.0, 1.0 ml of 0.1 M calcium chloride and 0.5 ml of 2% magnesium chloride. Tissues were incubated for 30 min at 4 C. After incubation, tissues were treated for 2 min in 2% lead nitrate according to the method of Wetzel et al. (1967).

Control tissues were incubated in a substrate-free medium, or in a substrate-medium containing 1 mM sodium fluoride (NaF) or 10 mM EDTA. Additional controls consisted of tissues heated for 1 min at 90 C prior to fixation in a complete substrate-medium.

Tissues were rinsed in buffer and post-fixed for 1 h at 4 C in 1% osmium tetroxide in 0.1 M sodium cacodylate. Specimens were dehydrated in a graded series of acetone- 30, 50, 70, 85 and 95% for 10 min and three changes of 10% acetone for 10 min each. Infiltration was carried out in epon embedding media mixed with acetone in ratios of 1:3, 1:2 and 1:1. Final embedding was carried out in epoxy resin and polymerization was for 24 h at 60 C.

Tissues were sectioned on a LKB Bromma 8800 Ultramicrotome III with glass knives and mounted on uncoated 300 mesh grids. Sections were viewed in a RCA EMU-4 Electron Microscope, and micrographs were taken at desirable magnifications.

Ultrastructural Localization of Acetylcholinesterase Activity in the Scolex

Three-month-old infections of the cysticercus of H. taeniaeformis were dissected from cysts present on the liver of male and female Long-Evans rats. The worms were relaxed in 0.85% physiological saline and the scolices were cut into small pieces. Tissues were fixed in 3% glutaraldehyde buffered in 0.1 M of sodium cacodylate at pH 7.4 for 2 h at 4 C. Following fixation, tissues were rinsed in buffer and then frozen in a cryostat. Frozen specimens were sectioned with a single razor blade at 50-60 mμ in diameter and incubated according to the method of Karnovsky and Roots (1964). The incubation medium consisted of 5 mg of the substrate, acetylthiocholine iodide, 6.5 ml of 0.1 M sodium hydrogen maleate buffer (pH 6.0), 0.5 ml of 0.1 M sodium citrate, 1.0 ml of 30 mM copper sulfate, 1.0 ml of distilled water, 1.0 ml of 5 mM potassium ferricyanide, and 1.5 g of sucrose. Incubation was carried out at 4 C for 30 min.

One group of control tissues was incubated in a substrate-free medium. A second group of tissues was heated at 90 C for 1 min prior to fixation and subsequently incubated in a

medium containing the substrate. Eserine, an inhibitor of cholinesterase activity, was placed in the incubation medium of a third group of tissues at a concentration of 10^{-4} M.

Tissues were rinsed in buffer and post-fixed in 1% osmium tetroxide for 2 h at 4 C in 0.1 M phosphate. Specimens were dehydrated in a graded series of acetone, infiltrated and embedded in epoxy resin. Sections were cut on a Bromma 8800 LKB ultramicrotome III and photographed in a RCA EMU-4 or Philips EM 100-C electron microscope.

CHAPTER IV

EXPERIMENTAL RESULTS

Macroscopic Studies

According to King and Showers (1969) the liver consists of two principal lobes, the right and left. The right consists of the right lobe proper, with the small quadrate lobe on the interior surface and the caudate lobe on the posterior surface. The line of demarcation between the right and left lobes is an indication of the anterior and superior surfaces by the falciform ligament, which passes from the liver to the diaphragm and the anterior abdominal wall. The liver occupies chiefly the upper right hypochondriac and epigastric regions beneath the diaphragm. Figure 1 is an uninfected liver from a Long-Evans rat. A three-month-old infection of the cysticercus of H. taeniaeformis in the liver of a rat consists of many cysts on all lobes of the liver as seen in Fig. 2. Figure 3 shows 102 cysts containing the cysticercus of H. taeniaeformis dissected from the liver of a Long-Evans rat.

Scanning Electron Microscopy

SEM of the cysticercus of H. taeniaeformis

Scanning electron microscopy has revealed many relevant components which characterize the tegumental surface of cestodes as seen with light and electron microscopy. An overall scan of the scolex reveals four suckers or acetabula

Fig. 1. Photomicrograph of an uninfected liver from a Long-Evans rat (x 1).

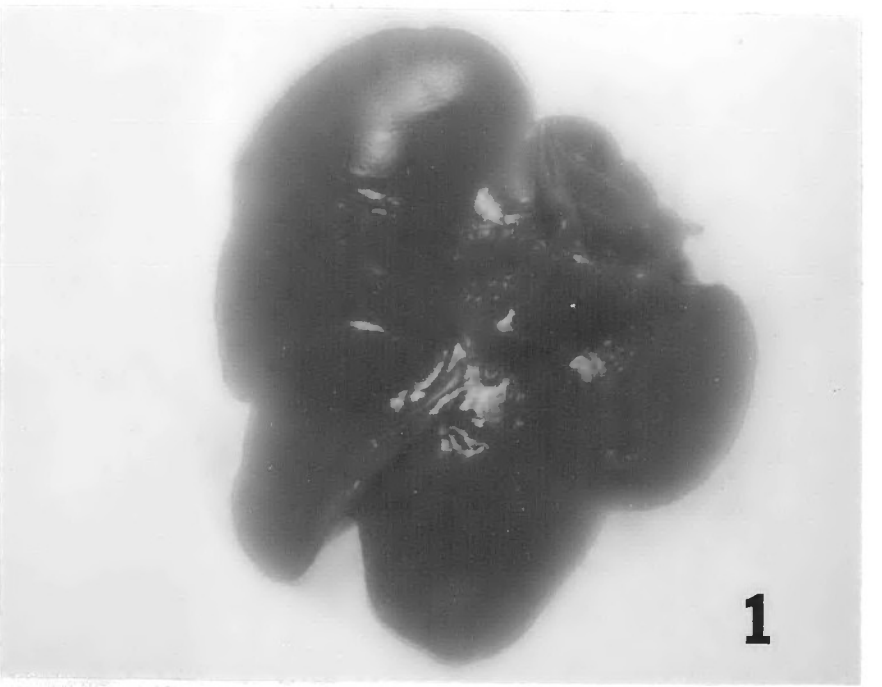
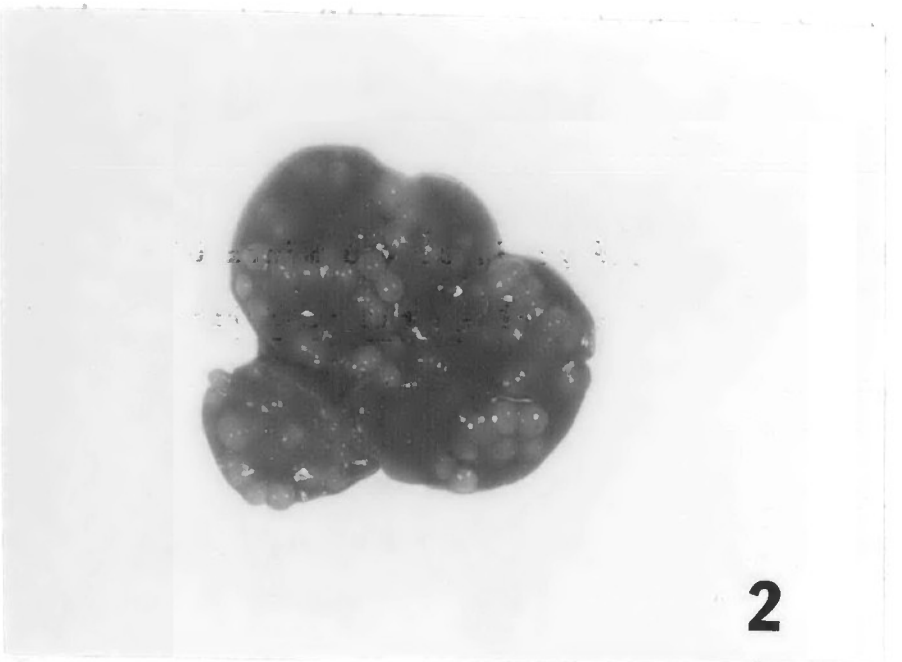
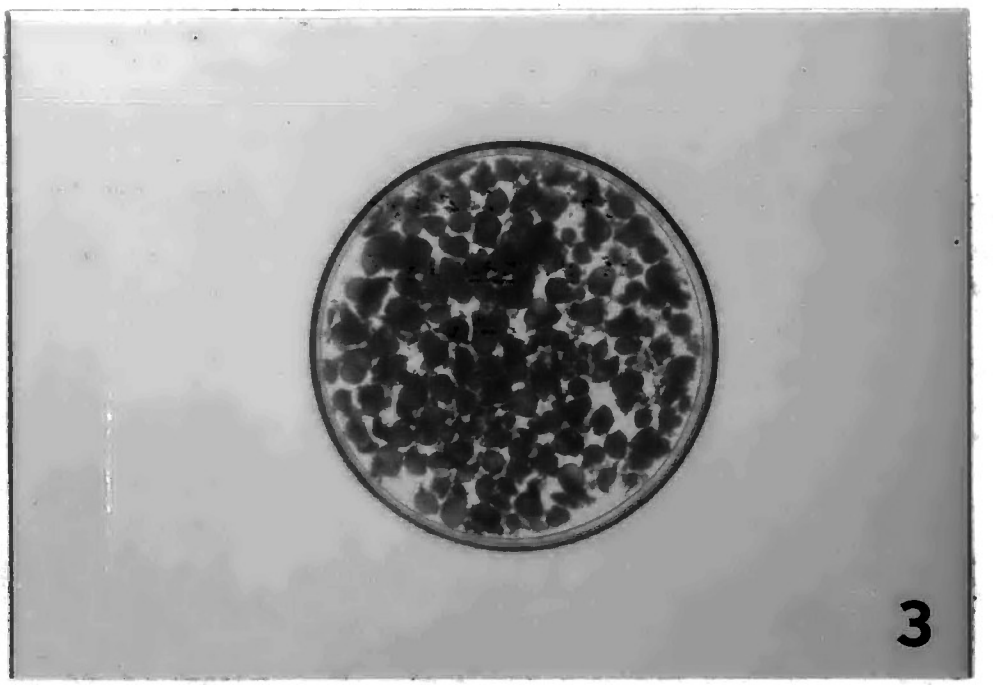

1

Fig. 2. Photomicrograph of a 3 month old infection of the cysticercus of H. taeniaeformis in the liver of a Long-Evans rat (x 1).



2

Fig. 3. Photomicrograph of 102 cysts containing the cysticercus of H. taeniaeformis dissected from the liver of a Long-Evans rat (x 1).


3

which are in a circle around the scolex, a rostellum which consists of a muscular cushion, rostellar sac and anterior and posterior rows of hooks. The anterior surface of the scolex is wrinkled and contains numeruous ridges and deep depressions. The suckers consist of many folds (Figs. 4-7). Figure 8 shows the surface of the scolex and the rostellum in a contracted or withdrawn state. When the rostellum is in the contracted state, it assumes the form of a deep cup-shaped structure into which the muscular cushion can be withdrawn. The muscular cushion of the rostellum consists of pit-like depressions and the rim of the rostellar sac consists of numerous compact folds (Fig. 9). At even a higher magnification, as seen in (Fig. 10), the cushion of the rostellum shows a network of thick fibers. An interior view of the scolex shows four suckers and a withdrawn rostellum (Fig. 11).

The anterior tip of the scolex with the rostellum in an expanded state is shown in Fig. 12. The anterior and posterior rows of hooks are uniform in size and protrude from large cavities. In the expanded state the rostellar sac has a biconvex shape. A higher magnification of the cushion of the rostellum reveals a rough pit-like surface and hooks along the rim of the rostellar sac (Fig. 13).

The inner and outer surface of the sucker consists of ridges, deep grooves and folds (Fig. 14). Large folds are more prominent on the outer surface. A higher magnification

Fig. 4. SEM of the cysticercus of H. taeniaeformis showing suckers (S), rostellum (R) and hooks (arrow) (x 150).

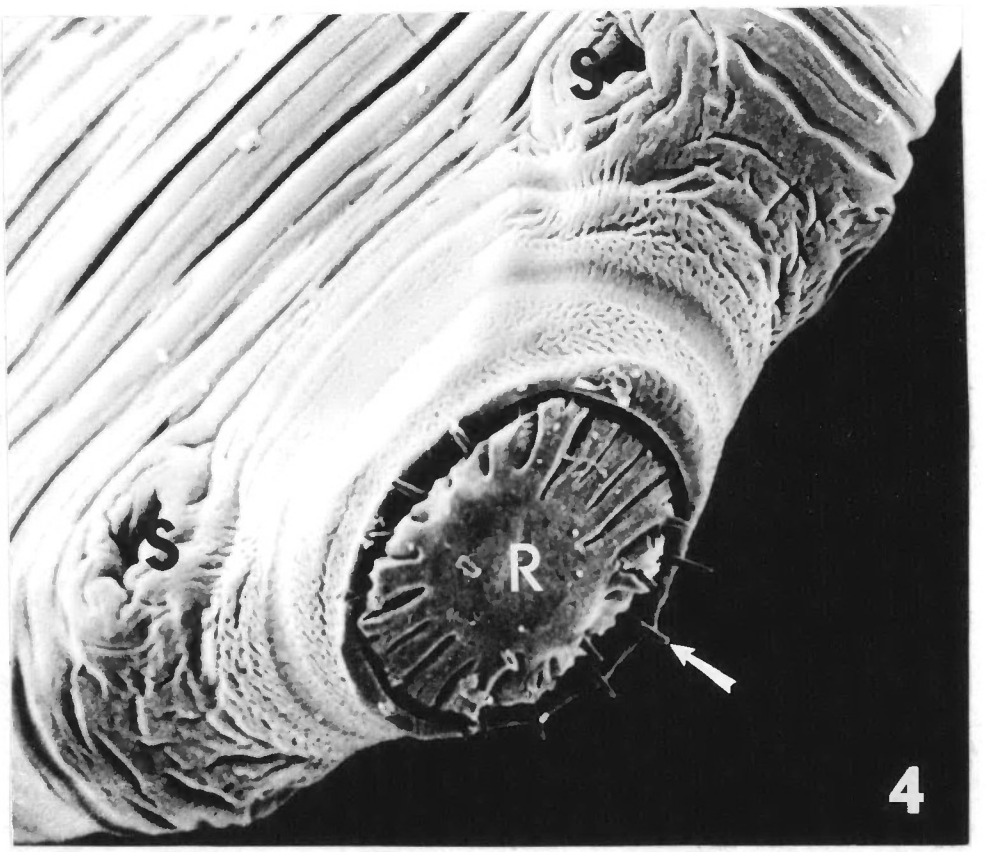

S
S
R
4

Fig. 5. SEM of scolex showing suckers (arrows), rostellum (R) and hooks (H) at a different angle (x 250).

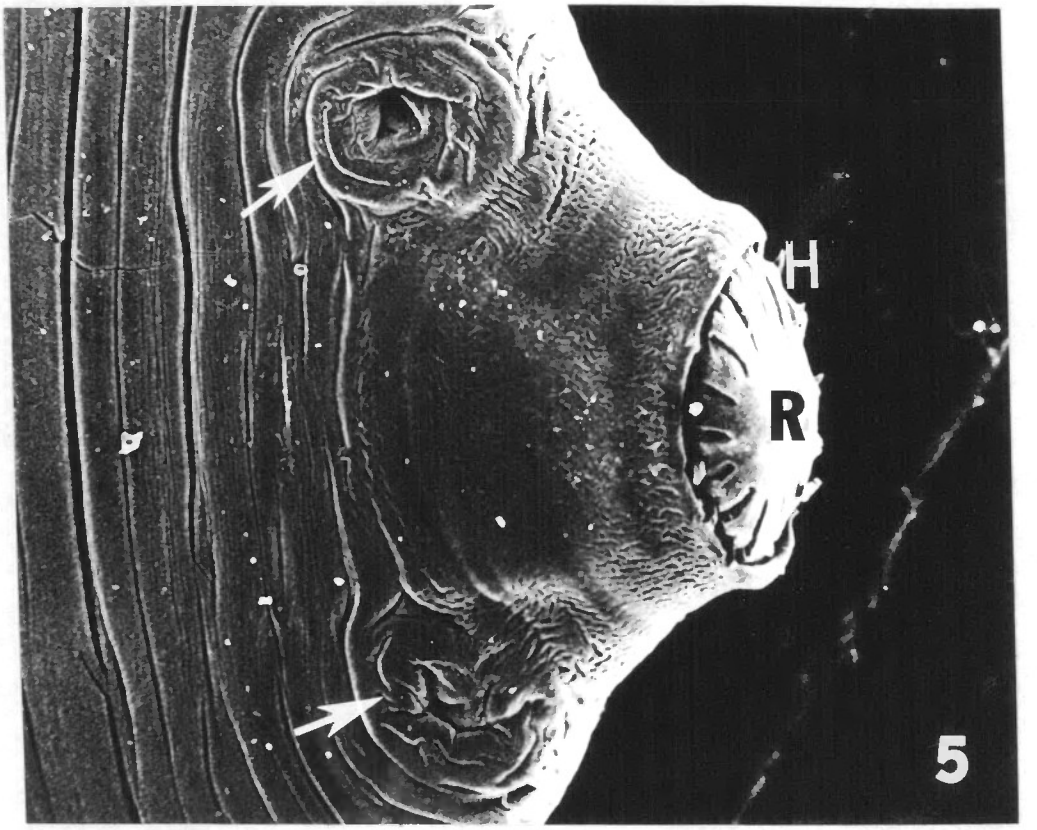

H
R
5

Fig. 6. Higher magnification of anterior tip of scolex showing irregular pattern of depressions (arrows) (x 300).

Fig. 7. SEM of scolex showing close-up of sucker (S) from lateral view (x 250).


S
7

Fig. 8. SEM of scolex showing contraction of rostellum and anterior surface (arrow) (x 350).

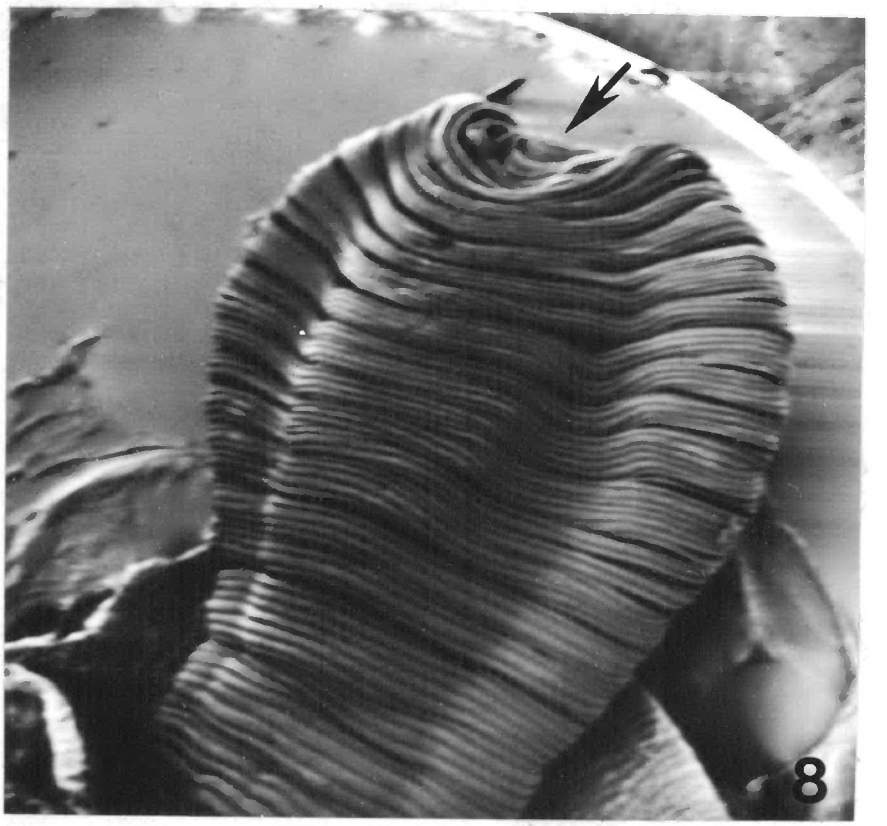

8

Fig. 9. Higher magnification of withdrawn muscular cushion of the rostellum (R) with pit-like depressions. The rostellum is surrounded by the rostellar sac (RS) (x 400).

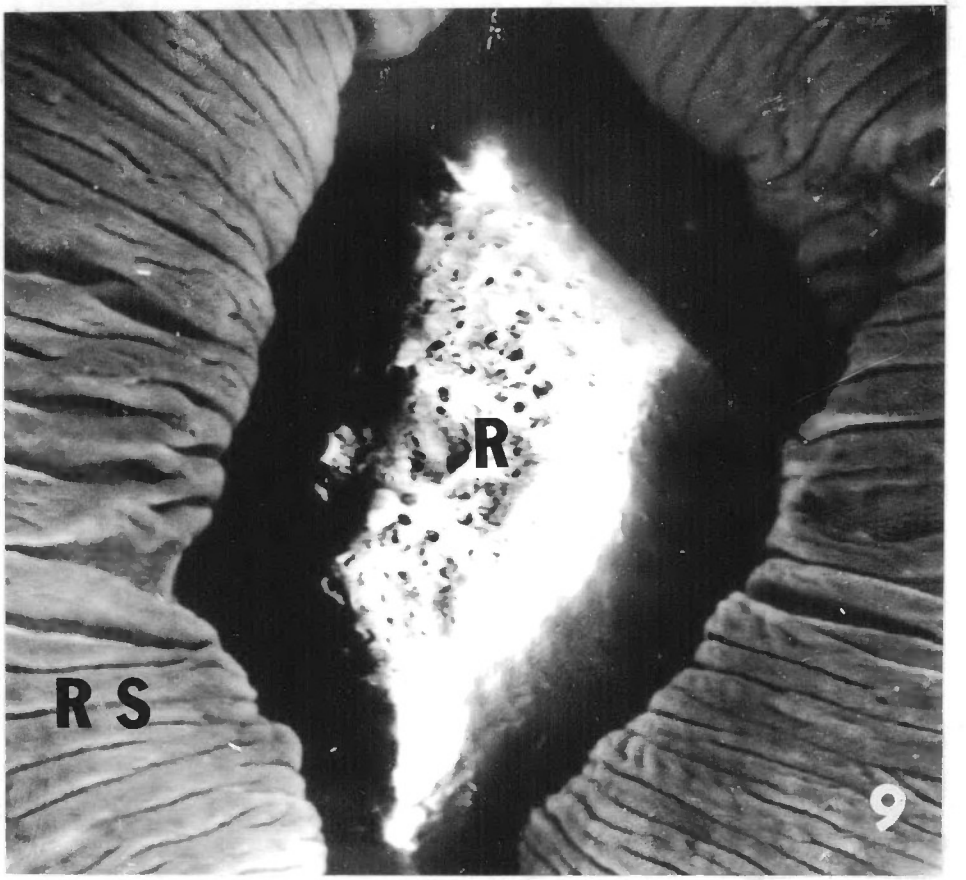

R
R S
9

Fig. 10. Higher magnification of muscular cushion showing network of thick fibers (x 1,000).

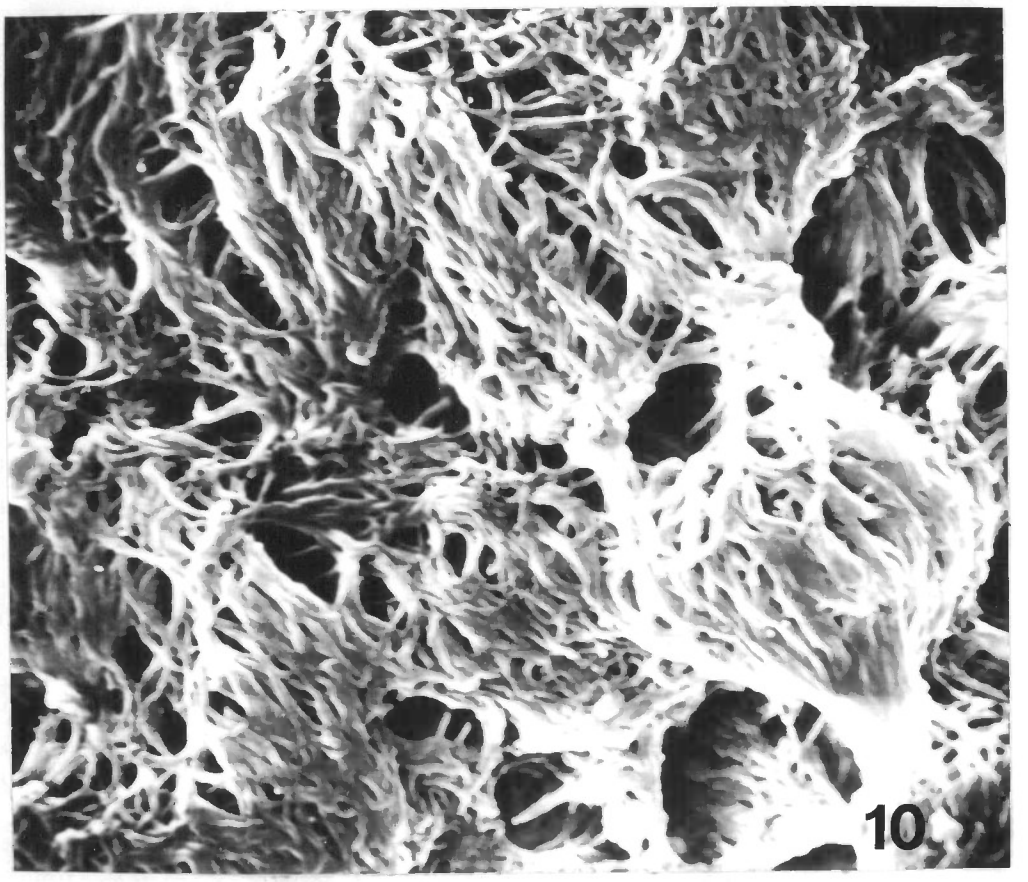

10

Fig. 11. SEM of scolex with contracted rostellum (R) showing muscular cushion (arrow) and suckers (S) (x 350).

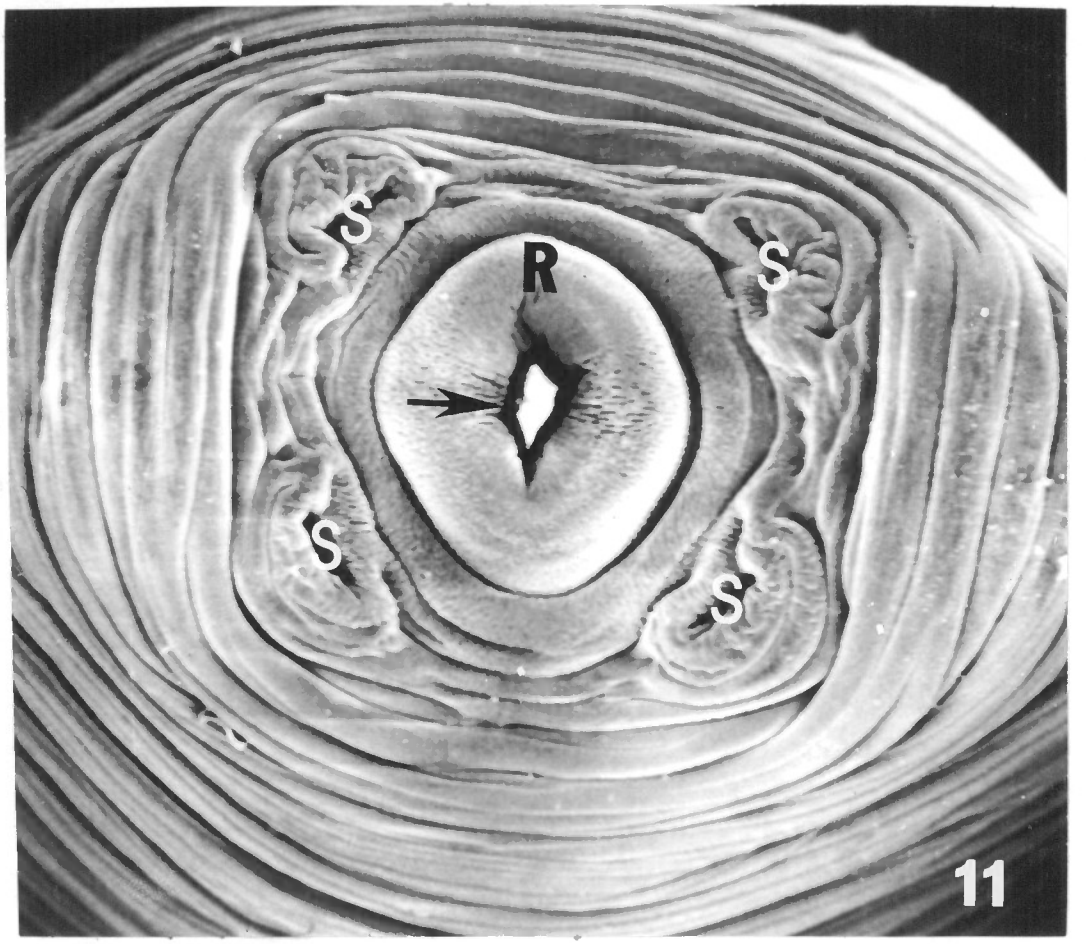

S
R
S
S
S
11

Fig. 12. SEM of anterior tip of scolex showing rostellum (R) with anterior and posterior rows of hooks (arrows) in the expanded state. Note biconvex shape of rostellar sac (RS) and depressions on the surface (x 350).

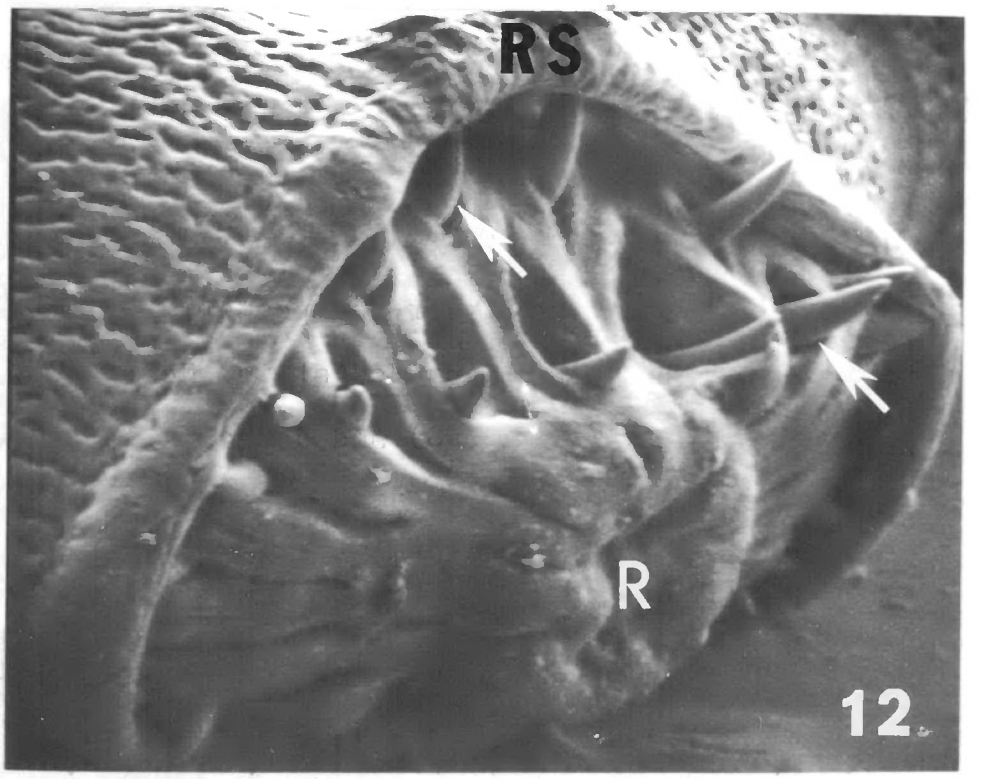

RS
R
12

Fig. 13. SEM of expanded muscular cushion (MC) of rostellum showing rim of rostellar sac (RS), hooks (H) and pitted surface (arrow) (x 500).

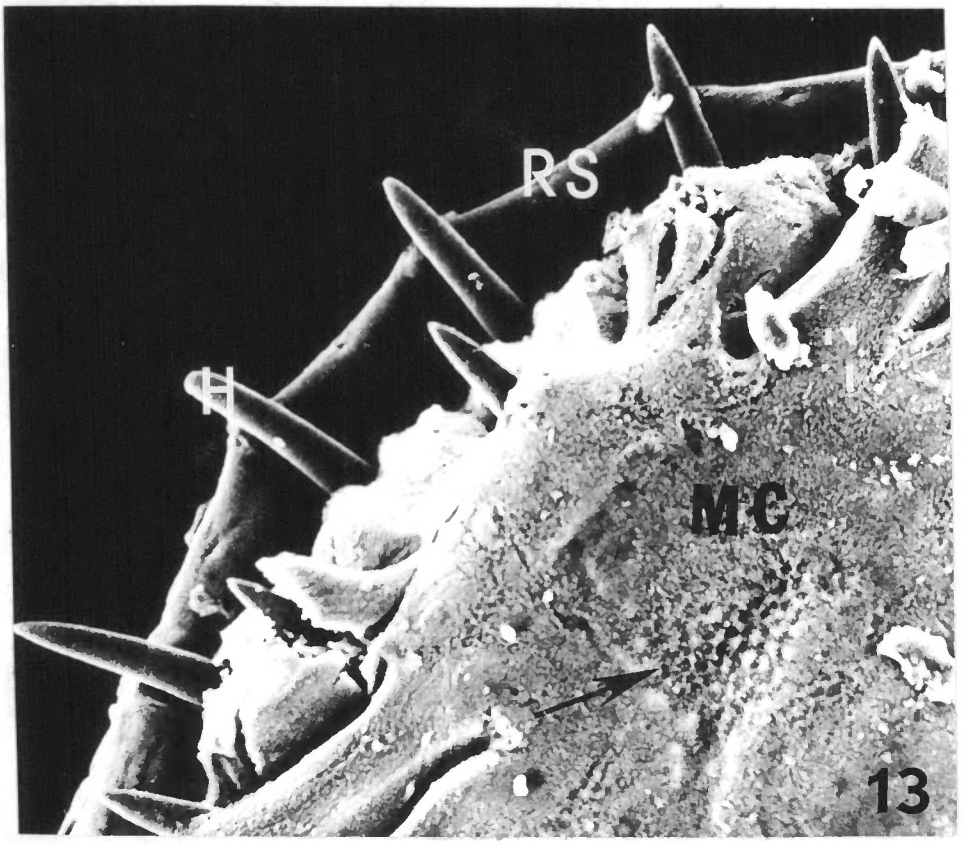
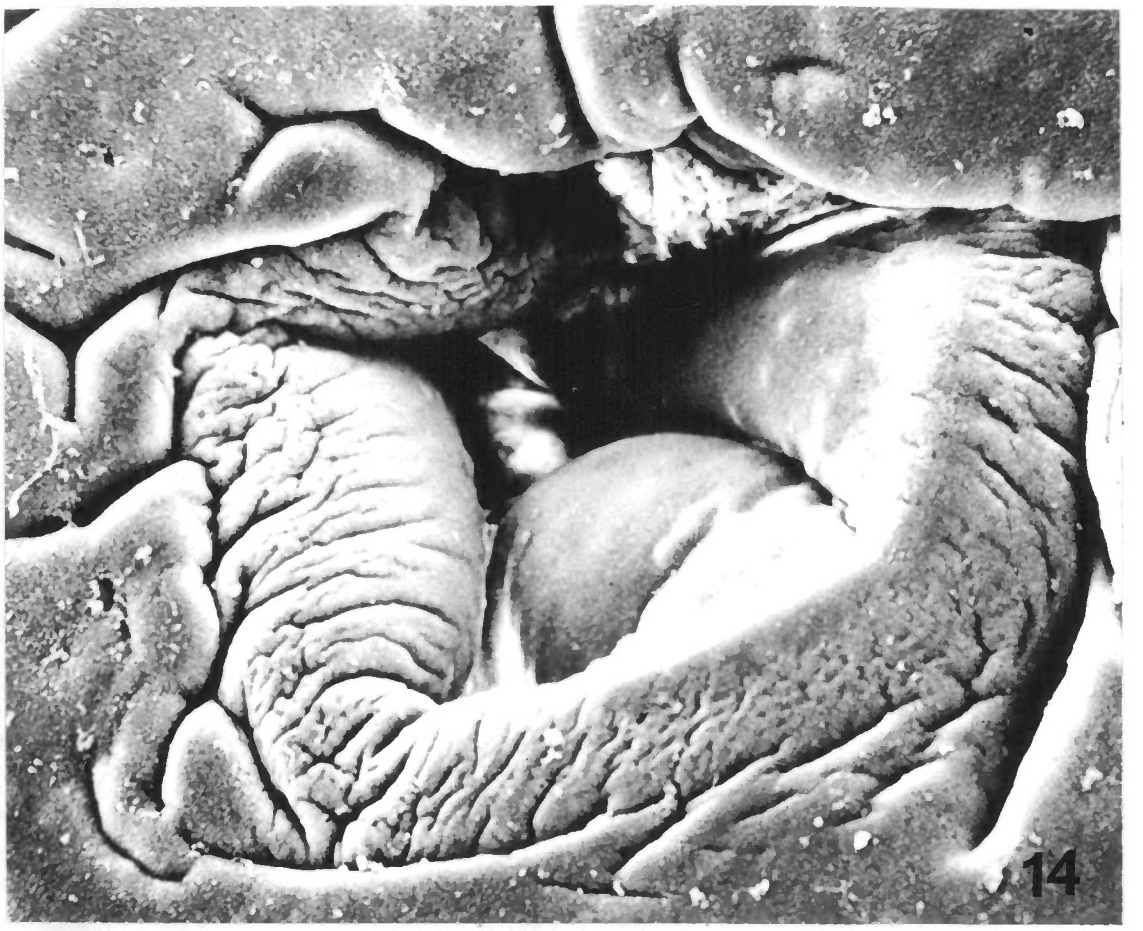

RS
MC
13

Fig. 14. SEM of sucker showing inner and outer surfaces with ridges and deep grooves (x 800).


14

of the inner and outer surface reveals the presence of microtriches (Fig. 15). The microtriches are of uniform size and are elevated. Deep elongated depressions can be seen among them (Figs. 16, 17). A close-up of a different sucker shows numerous compact folds and grooves (Fig. 18).

The strobila is composed of proglottids of uniform size (Fig. 19). At higher magnifications irregular masses of proglottids may be found in between other proglottids. The surface is roughened with prominent bosses of uniform size and distribution (Fig. 20). At even higher magnification the microtriches become apparent (Fig. 21).

Examination of the surface and posterior end of the bladder at low magnifications reveals a surface with irregularly arranged depressions and ridges also studded with densely packed bosses of uniform size and distribution (Figs. 22 and 23). At higher magnification the lateral surface of the bladder shows irregularly arranged openings which are presumably excretory pores. The microtriches are revealed as compact and dense structures (Fig. 24). Figure 25 is a close-up view of the microtriches and a lateral pore highly magnified. When the surface of the tegument is fractured transversely, the cells of the tegument are oval to spherical in morphology, and at low magnifications show a smooth surface (Fig. 26).

SEM of the Eggs of Hydatigera taeniaeformis

An electron scan of the eggs of H. taeniaeformis at high

Fig. 15. Higher magnification of inner and outer surface of sucker showing microtriches (arrows) (x 1,150).


15

Fig. 16. Close-up of sucker showing microtriches (M) (x 7,100).

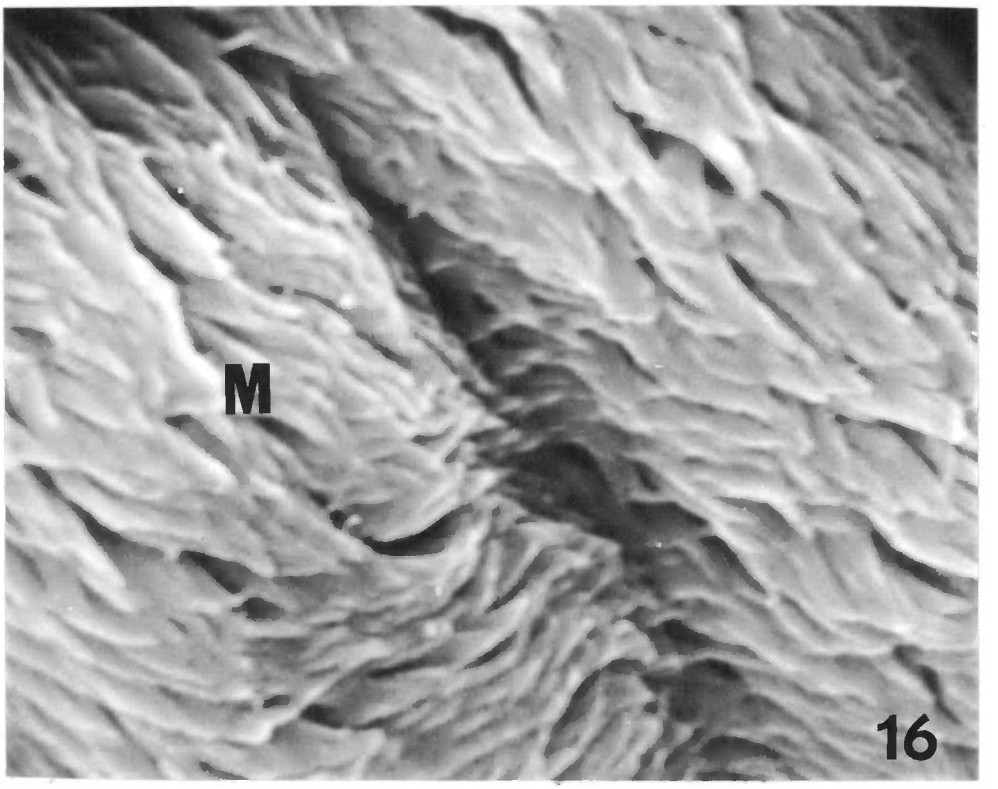

M
16

Fig. 17. Higher magnification of microtriches (M) (x 10,100).

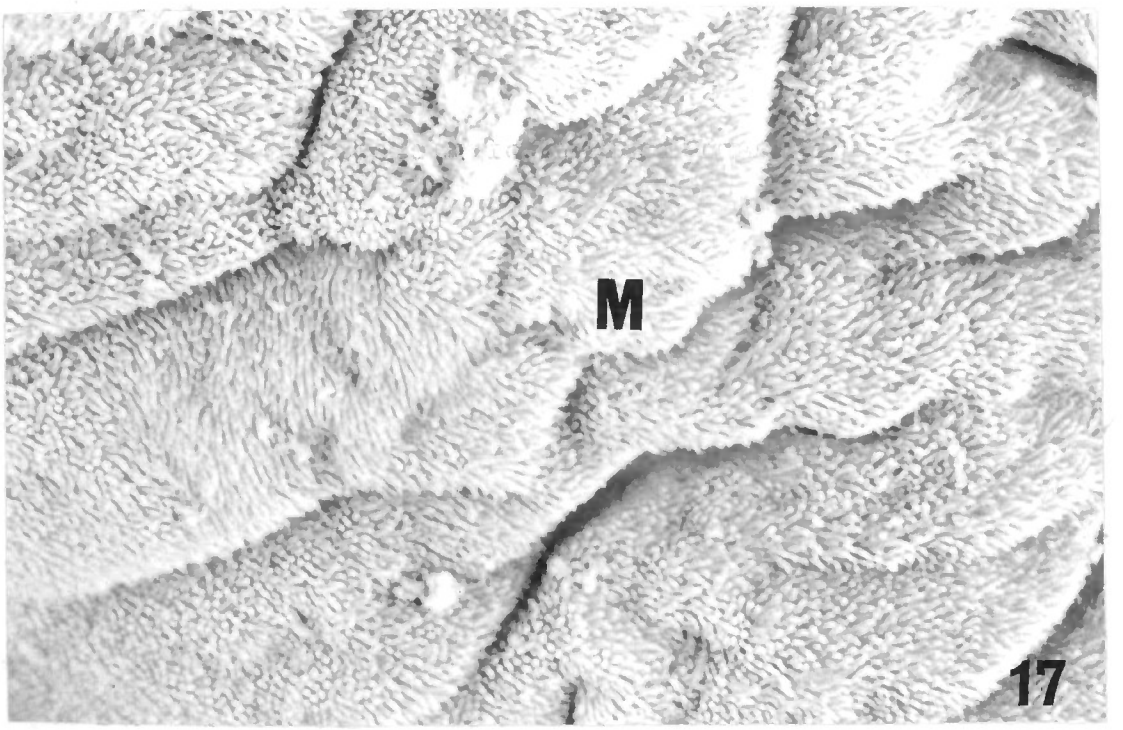

M
17

Fig. 18. SEM of a different sucker showing compact folds (F) and deep grooves (arrow) (x 1,100).


F
18

Fig. 19. SEM of strobila showing proglottids (P) (x 200).

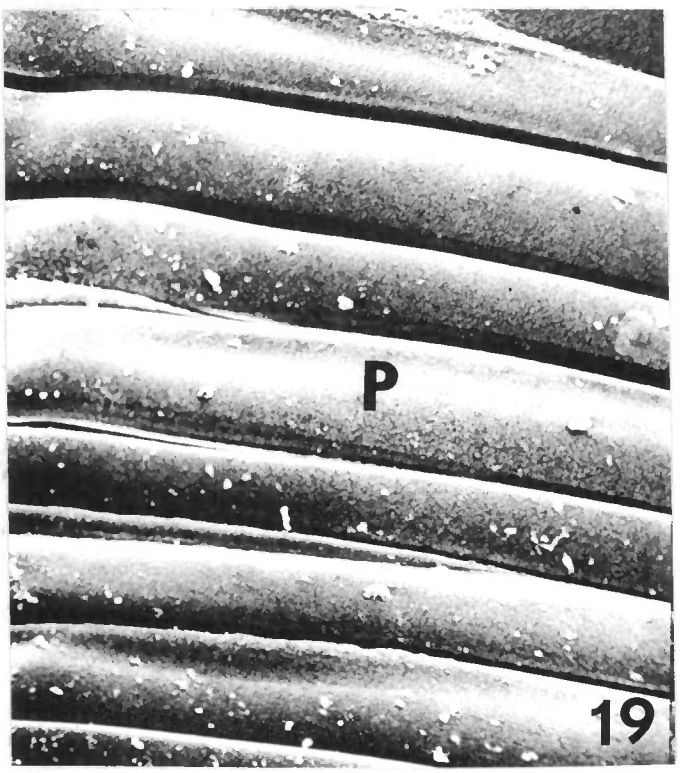

P
19

Fig. 20. Higher magnification of proglottids (x 1,000).


20

Fig. 21. Higher magnification of proglottids showing microtriches (arrows) (x 1,100).

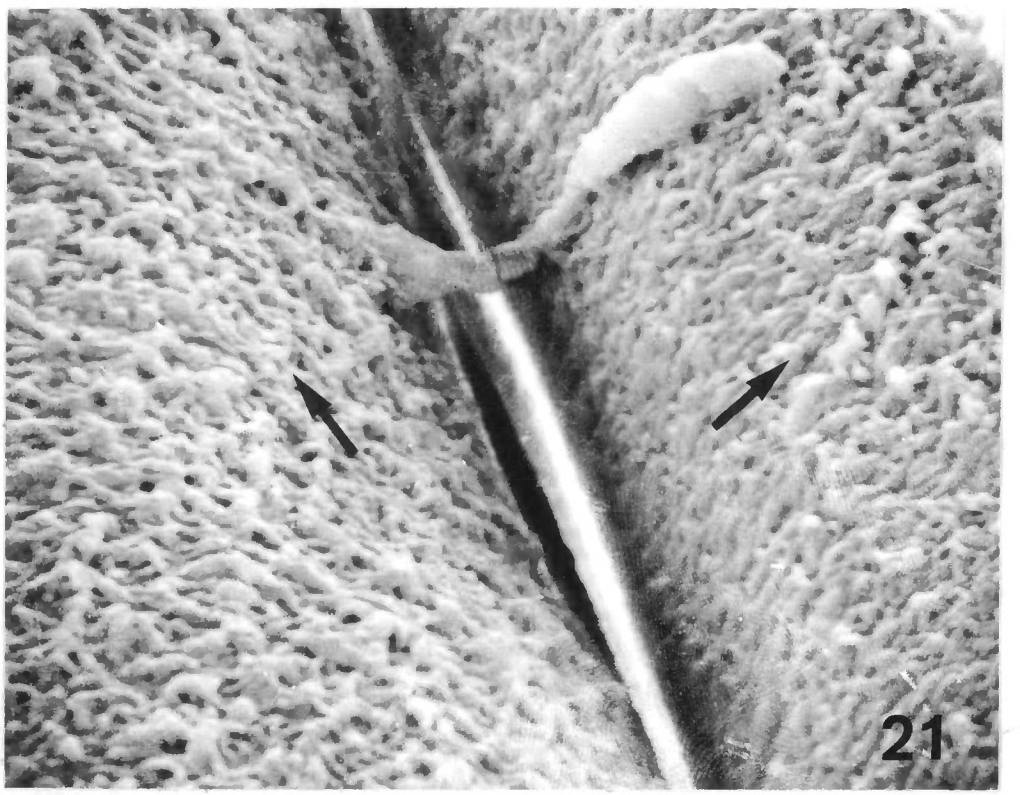

21

Fig. 22. SEM of pear-shaped bladder (B) (x 40).

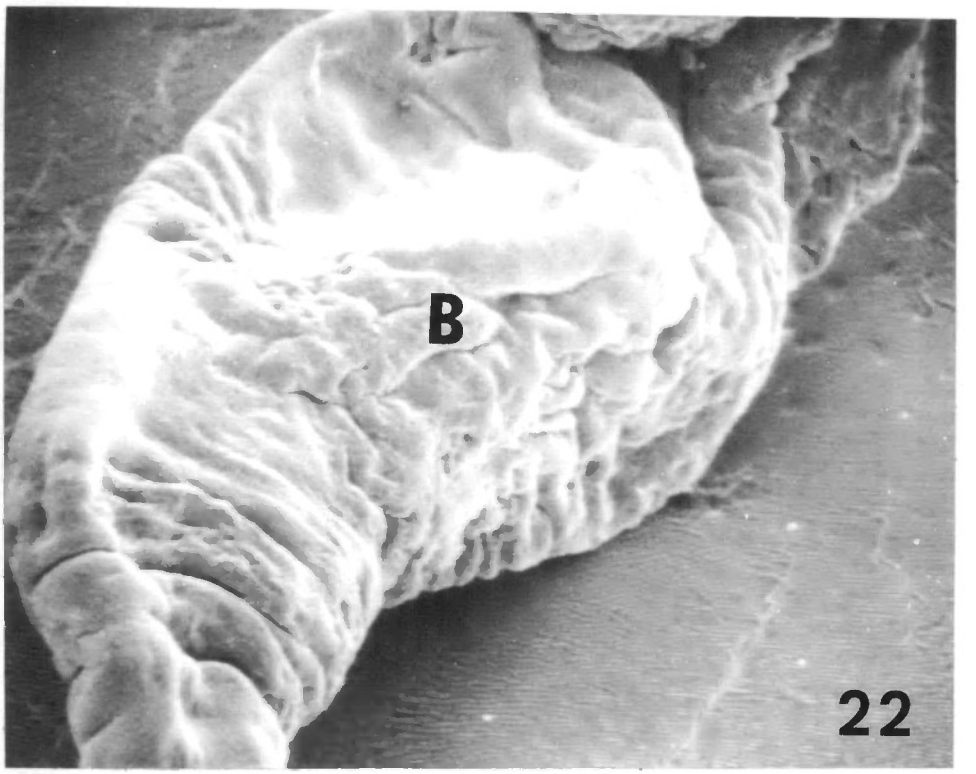

B
22

Fig. 23. Posterior tip of bladder (arrow) (x 500).

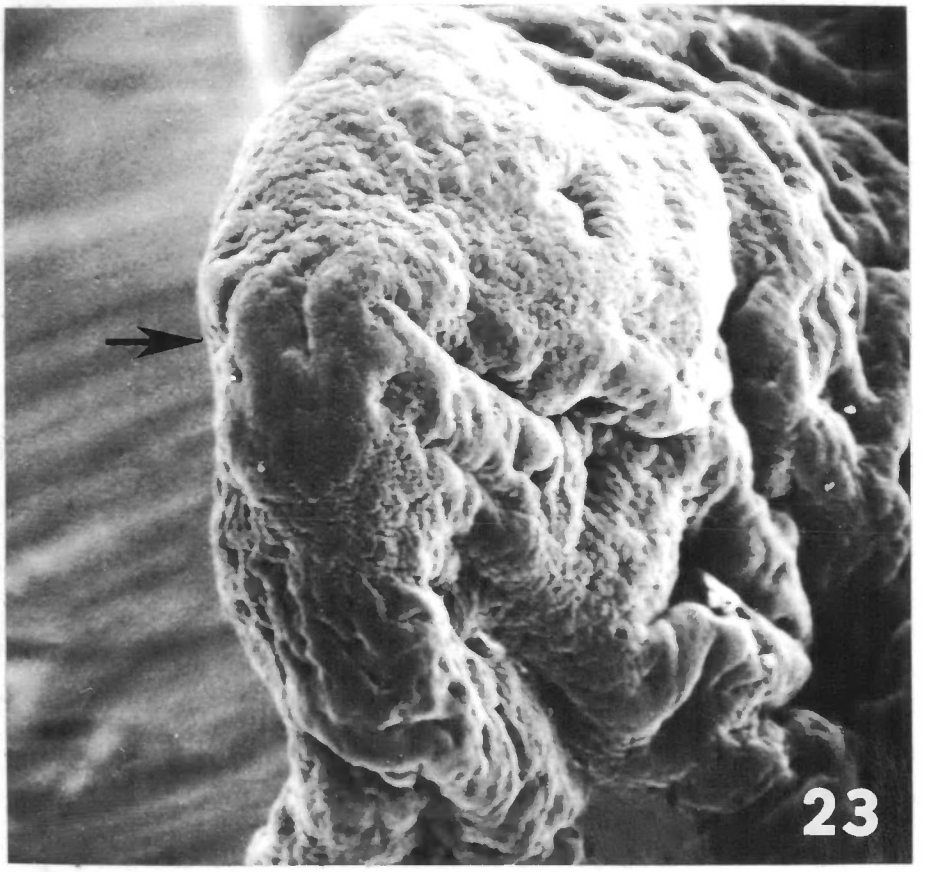

23

Fig. 24. Higher magnification of bladder showing microtriches (M) and lateral excretory pores (arrows) (x 10,100).

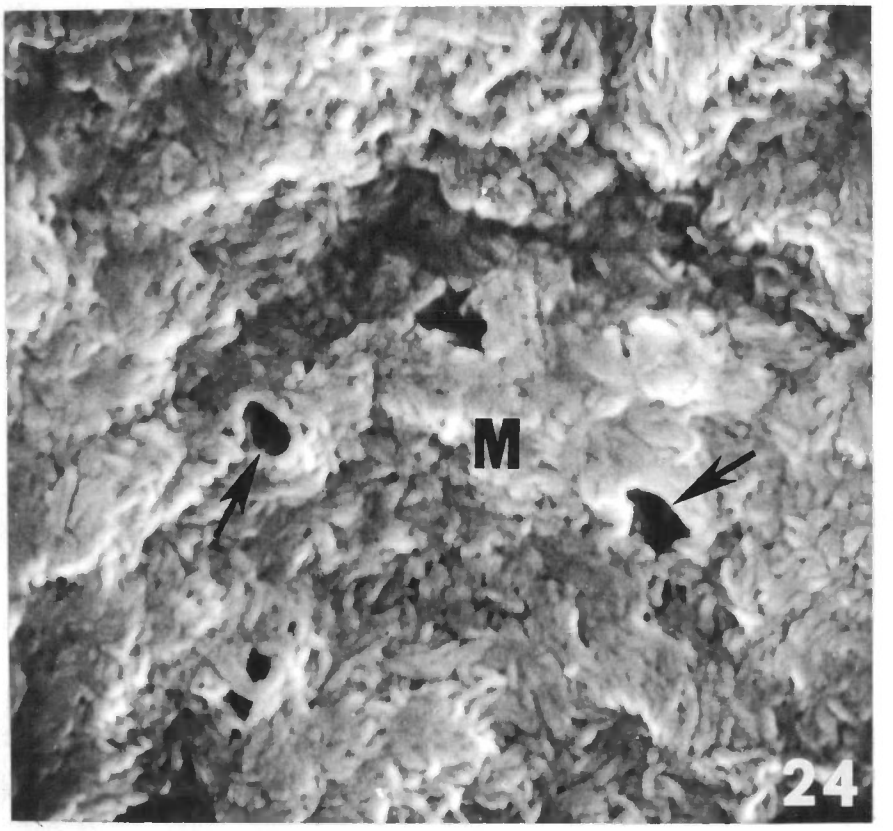

M
24

Fig. 25. Close-up of microtriches and lateral excretory pore (x 16,200).

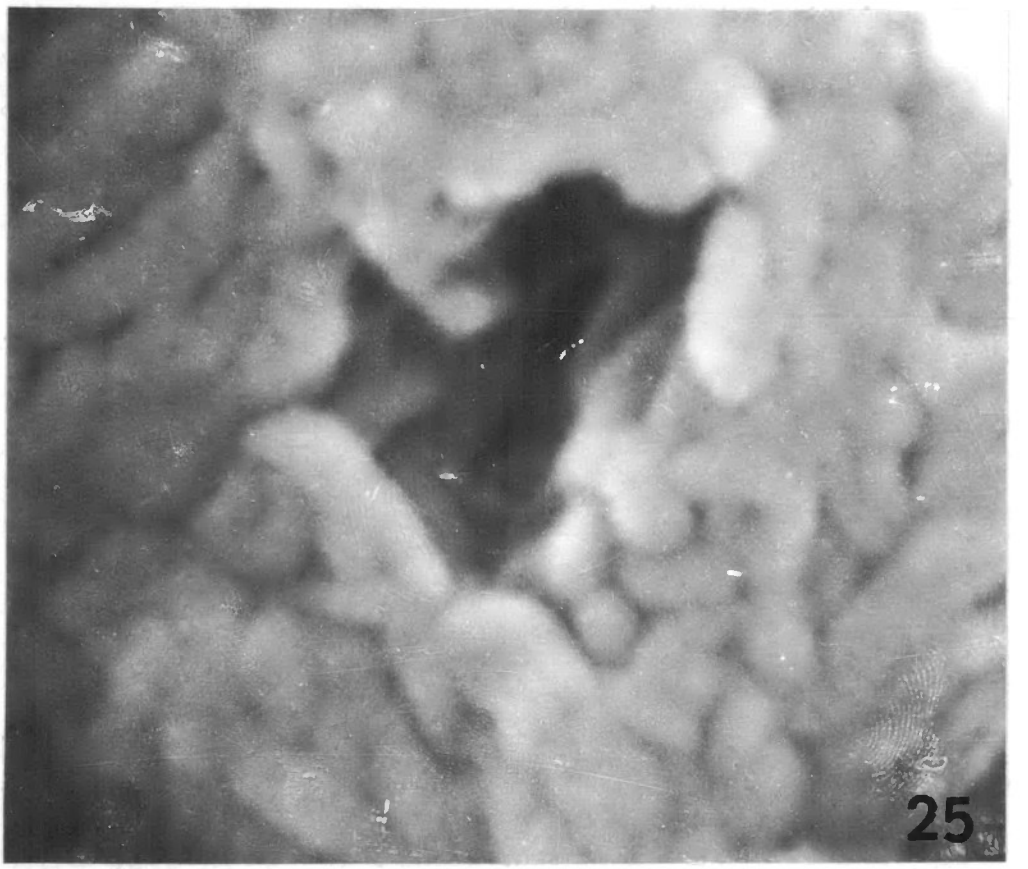
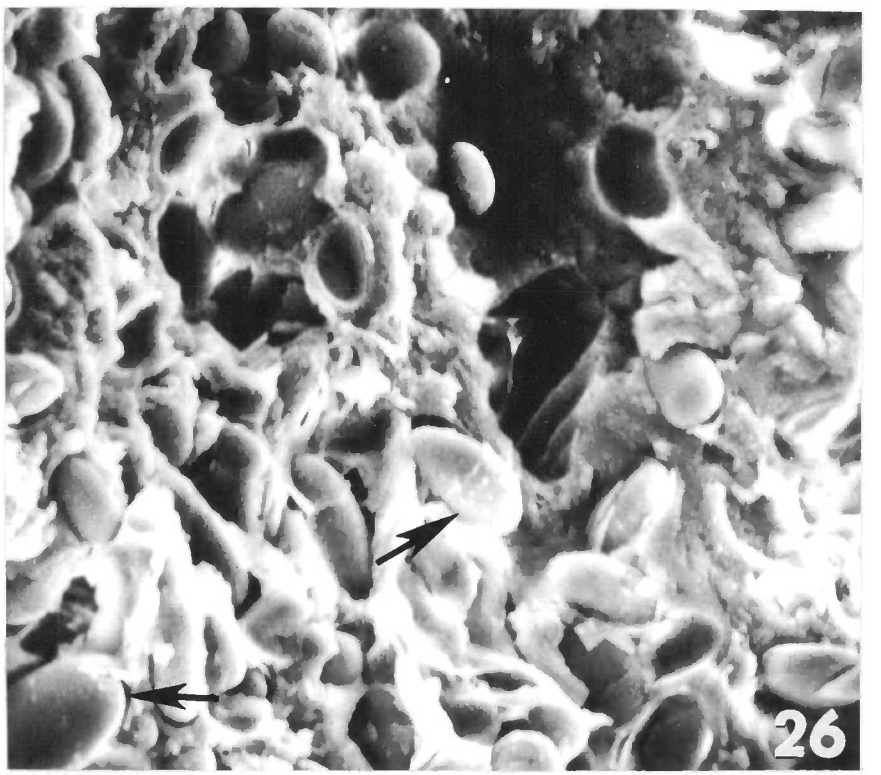

25

Fig. 26. SEM of inside of tegument showing inner cells (arrows) (x 700).


26

magnifications revealed certain characteristics of the eggs that could not be seen at lower magnifications. The eggs show a smooth surface (Fig. 27, and when they are treated with high concentrations of EDTA, the surface is distorted. Figure 28 shows the effects of EDTA on the egg surface and the emerging hooks of the embryo.

When an egg that is not subjected to high concentrations of EDTA is fractured, the inner surface membrane shows irregular ridges and depressions, too, the outer surface of the egg is not smooth (Fig. 29). At higher magnification, the nature of the inner surface of the egg is more apparent. The outer surface of the egg shows a scale-like sculpture with denticles irregular in size and distribution (Fig. 31). A further view of the egg shows a scale-like outer surface with denticles and another smooth membrane beyond the membrane with rough-like ridges and depressions (Fig. 31). Figure 32 shows a highly magnified view of the outer surface. The scale-like pattern is irregularly distributed over the entire surface of the egg. Small, spherical bosses can be seen in some areas of the egg surface. A different egg, possibly at an earlier stage of development, exhibits surface characteristics of pit-like depressions and small, spherical bosses irregularly distributed over the surface (Fig. 33).

Fig. 27. SEM of eggs of H. taeniaeformis (x 1,000).

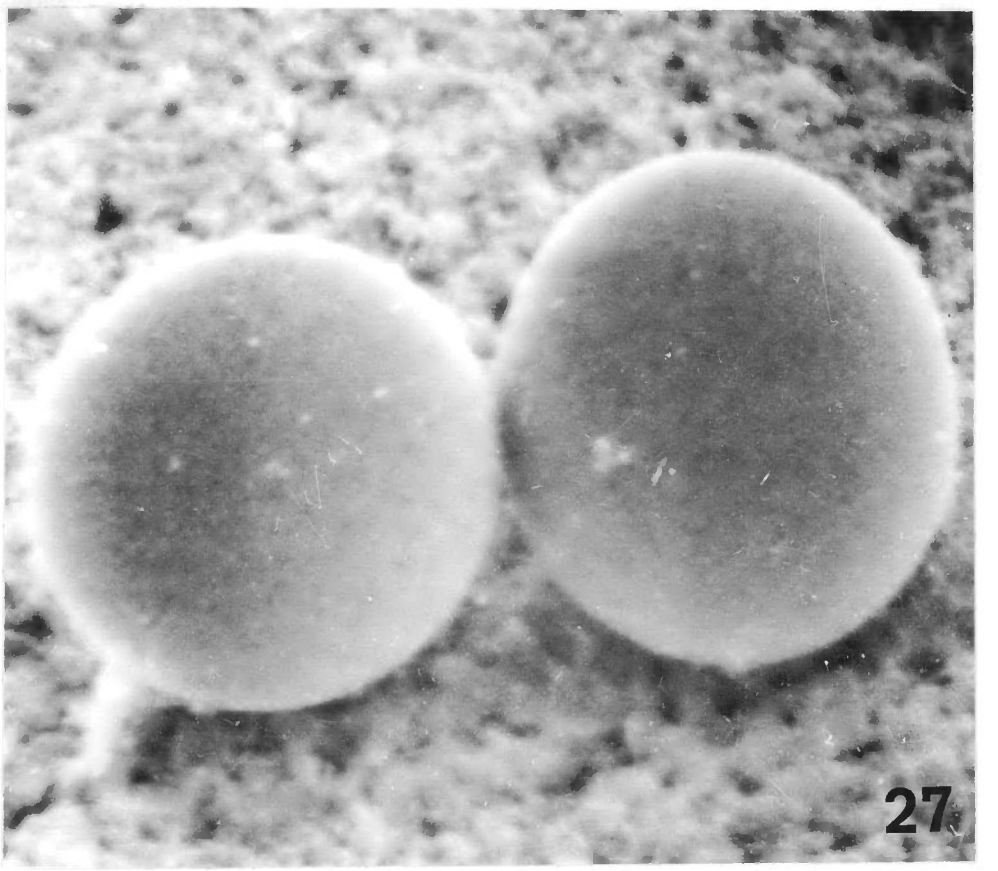

27

Fig. 28. Egg showing emerging hooks of embryo (arrow). Note distortion of surface due to EDTA (x 6,500).

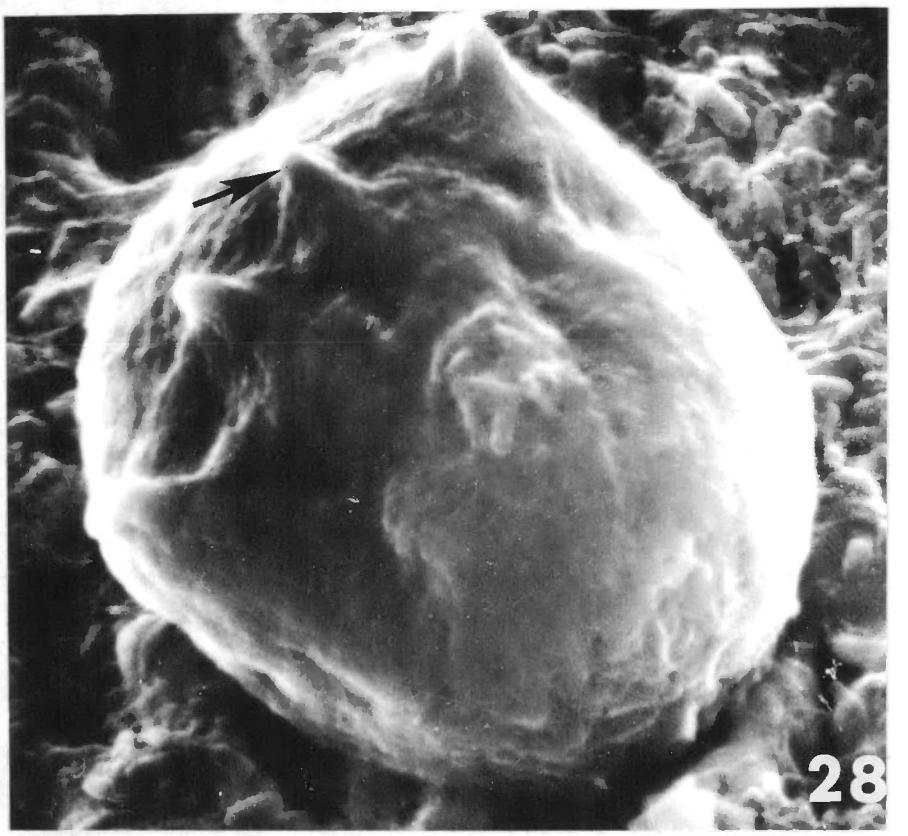

28

Fig. 29. Fractured egg showing inner membrane surface (arrow) (x 3,500).

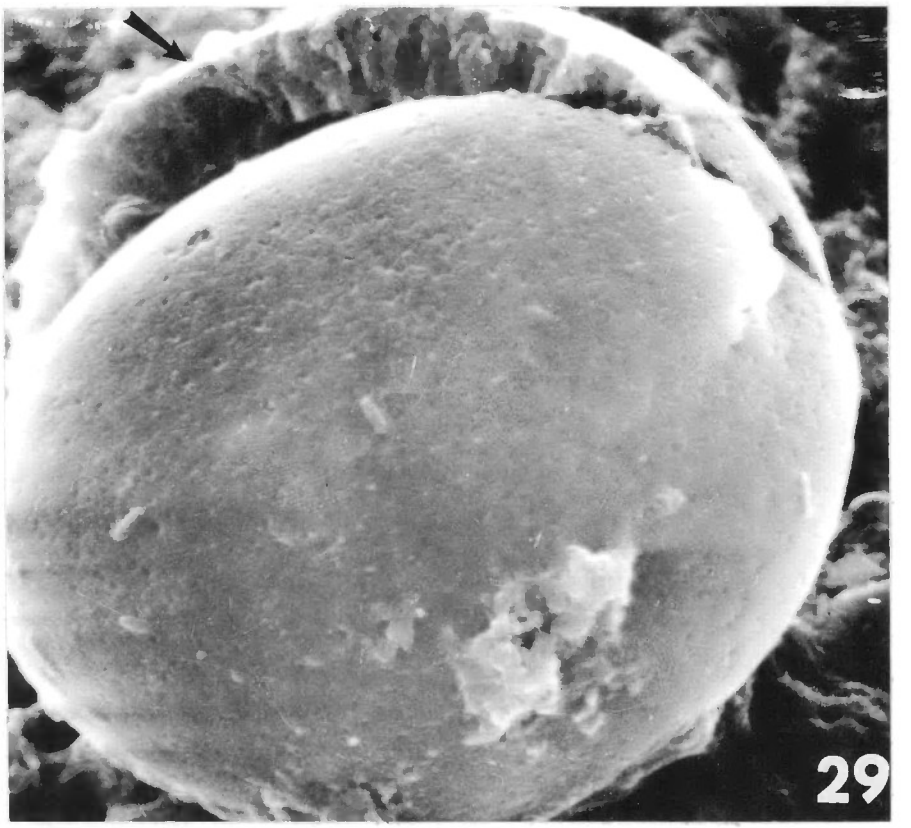

29

Fig. 30. Close-up of fractured egg showing inner membrane surface (arrow) and outer scale-like surface (SS) (x 15,000).

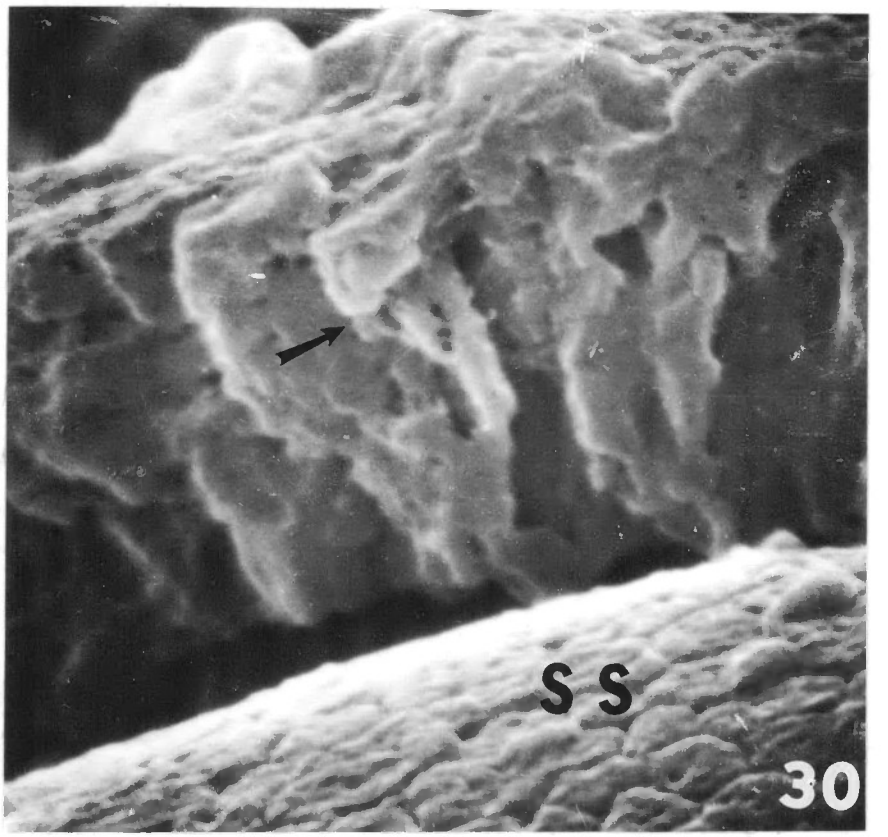

S S
30

Fig. 31. Close-up of outer surface of fractured egg showing scale-like surface (SS) and another inner membrane (arrow) (x 65,000).

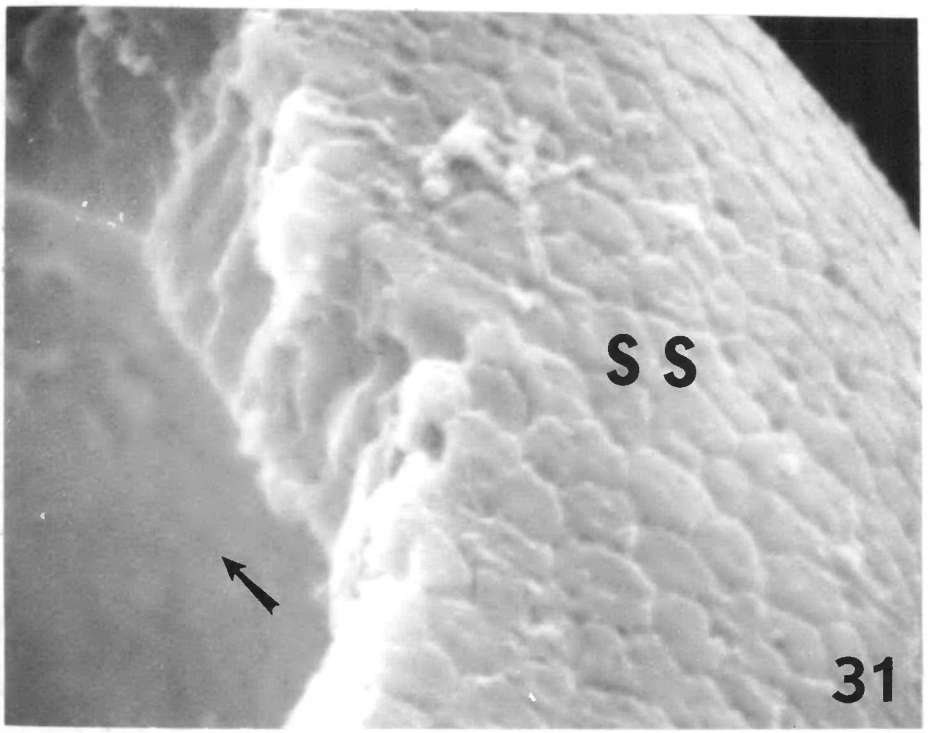

S S
31

Fig. 32. Close-up of scale-like surface with bosses (arrows) in some areas (x 68,000).

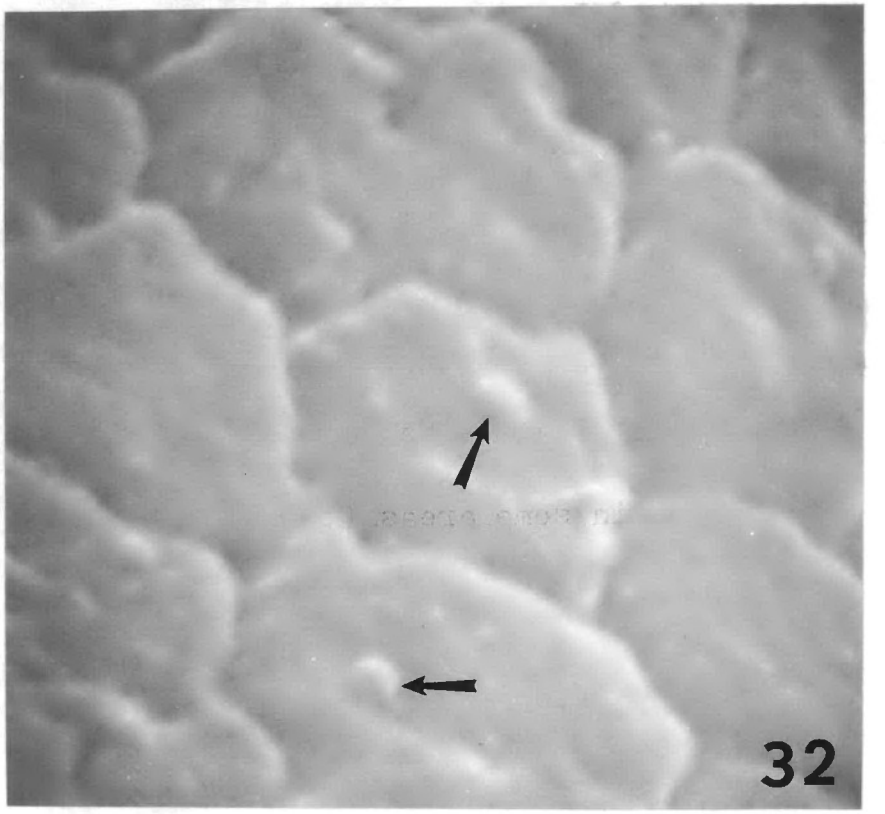
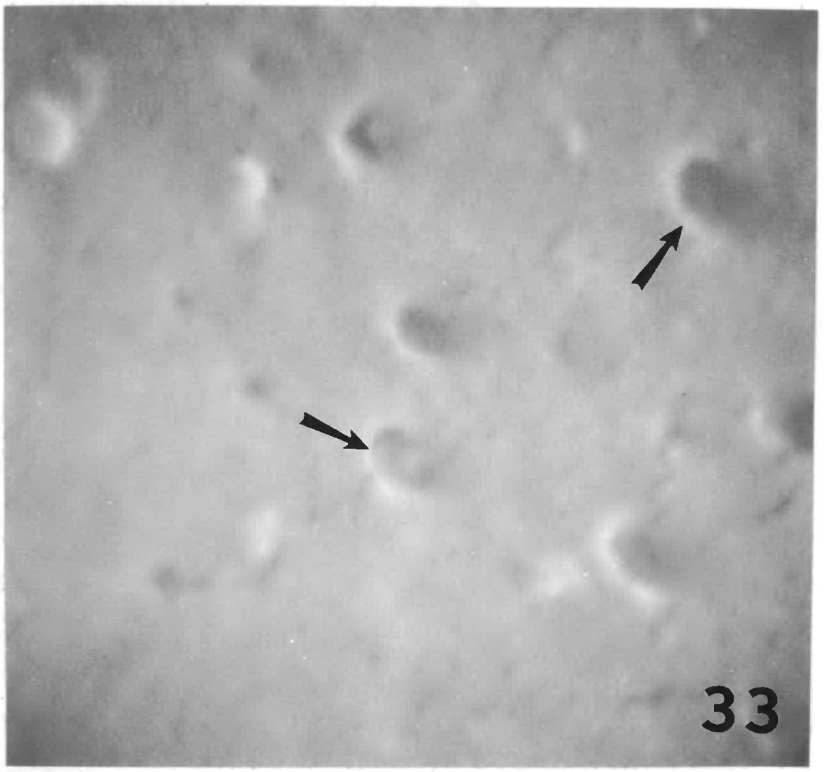

32

Fig. 33. SEM of egg showing pit-like depressions (arrows) (x 68,000).


33

Transmission Electron Microscopy

Ultrastructural Localization of Alkaline Phosphatase Activity in the Tegument

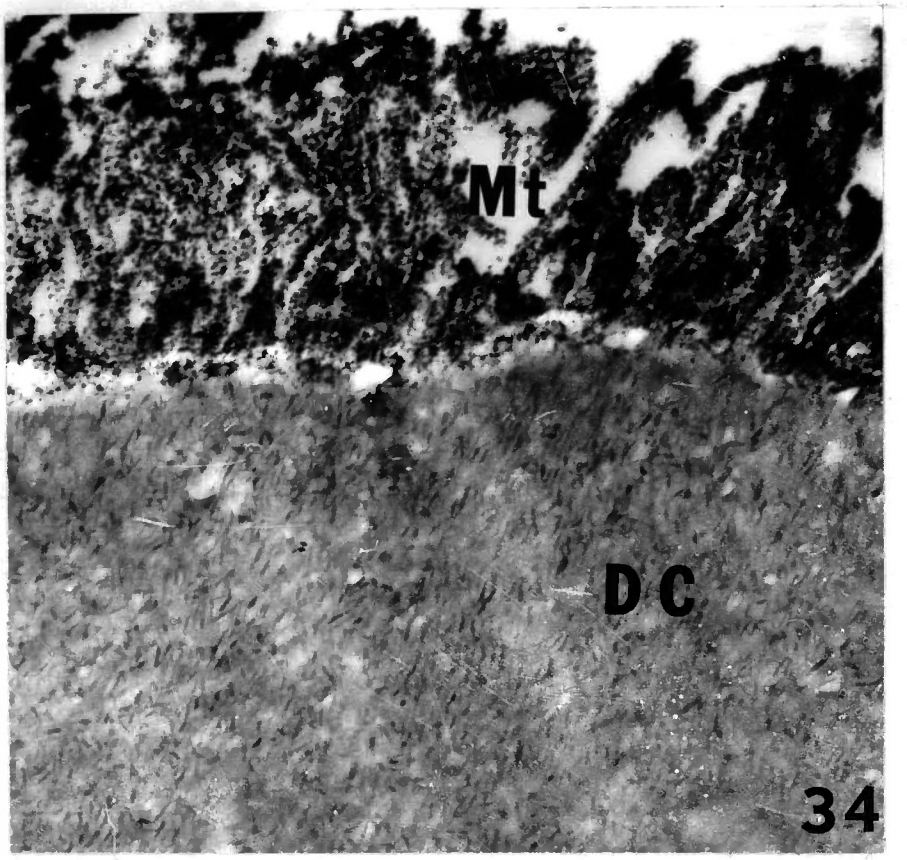
The ultrastructural localization of alkaline phosphatase activity was demonstrated in the tegument of the cysticercus of H. taeniaeformis using the Gomori procedure, as modified by Wetzel et al. (1967). A black precipitate in the form of electron dense granular deposits was taken as an indication of enzyme activity. A very strong reaction was observed on the microthrix brush border of sections incubated in a medium containing the substrate, beta-glycerophosphate. The distal cytoplasmic region showed an absence of alkaline phosphatase activity (Fig. 34). Tissues incubated in a medium without the substrate showed an absence of enzyme activity (Fig. 35).

Alkaline phosphatase activity was inhibited on the microthrix brush border when 1 mM NaF was placed in the incubation mixture. The lead precipatate revealed an interesting form with the NaF inhibition. Small, spherical, doughnut-shaped structures about 0.1 in diameter were observed (Fig. 36). When 10 mM of another inhibitor, EDTA was used, partial inhibition was observed on the microthrix brush border (Fig. 37). Enzyme activity was inhibited to some degree in all sections examined when 1 mM NaF or 10 mM EDTA was used.

The strobila tegument of worms heated 1 min prior to fixation showed a complete absence of alkaline phosphatase

Fig. 34. Alkaline phosphatase activity on microthrix border (Mt), unstained (x 24,000). Distal cytoplasm (DC).

Fig. 35. No-substrate control. Note absence of alkaline phosphatase activity on microthrix border (arrow), unstained (x 20,000). Distal cytoplasm (DC); Perinuclear cytoplasm (PC).


Mt
DC
34

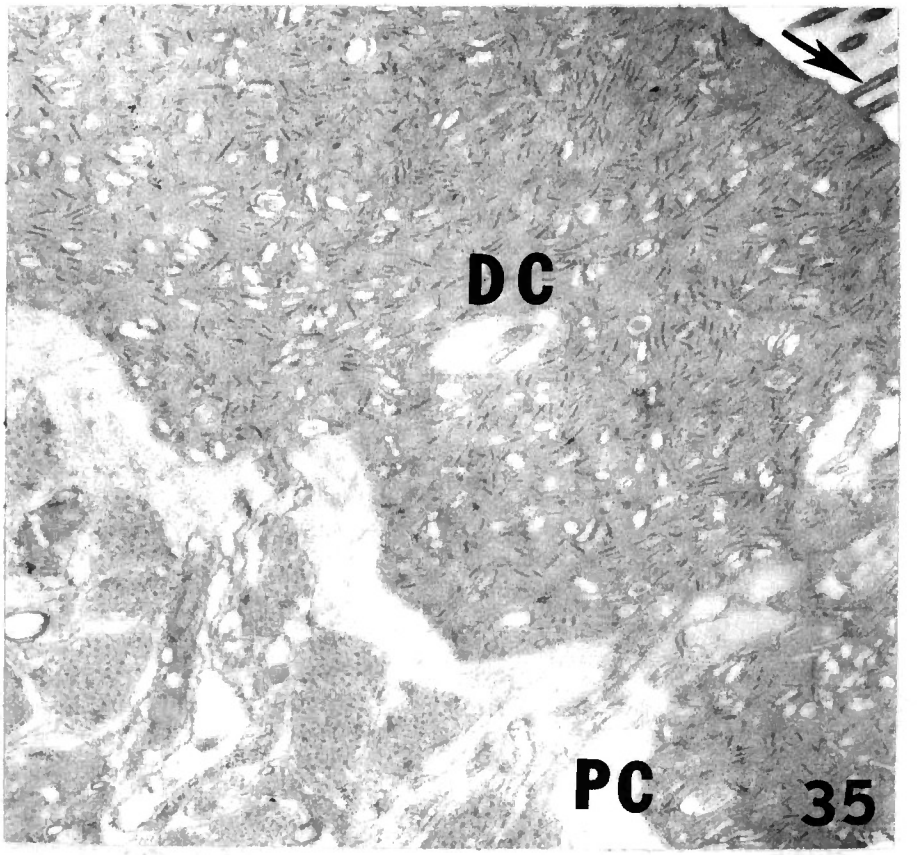
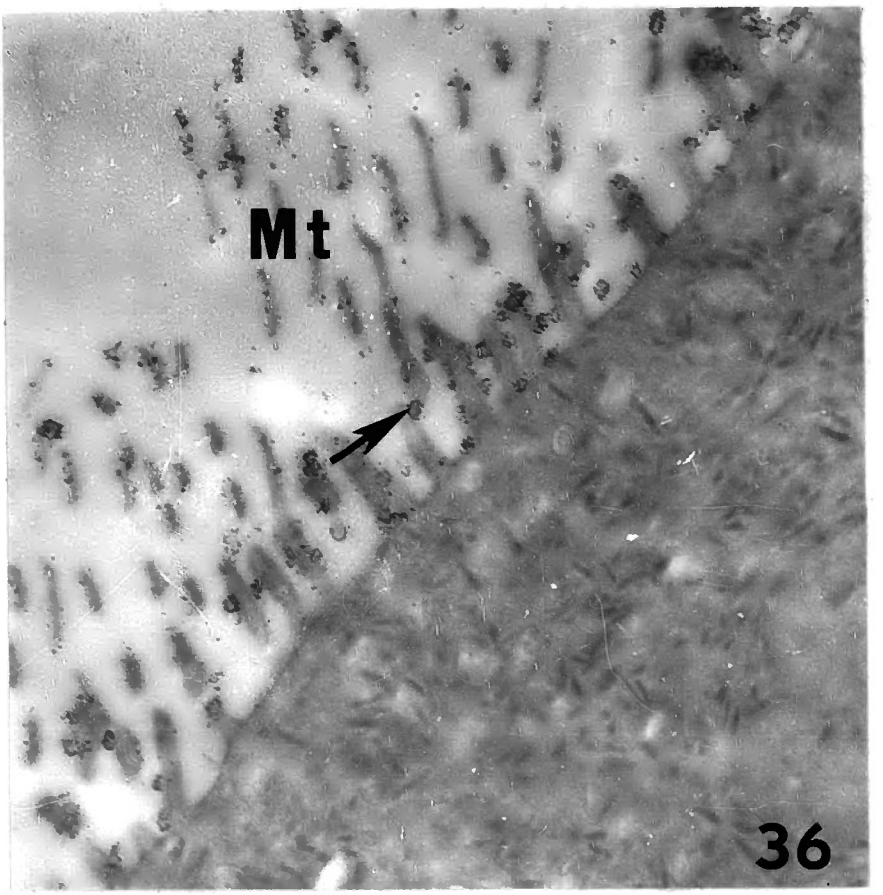
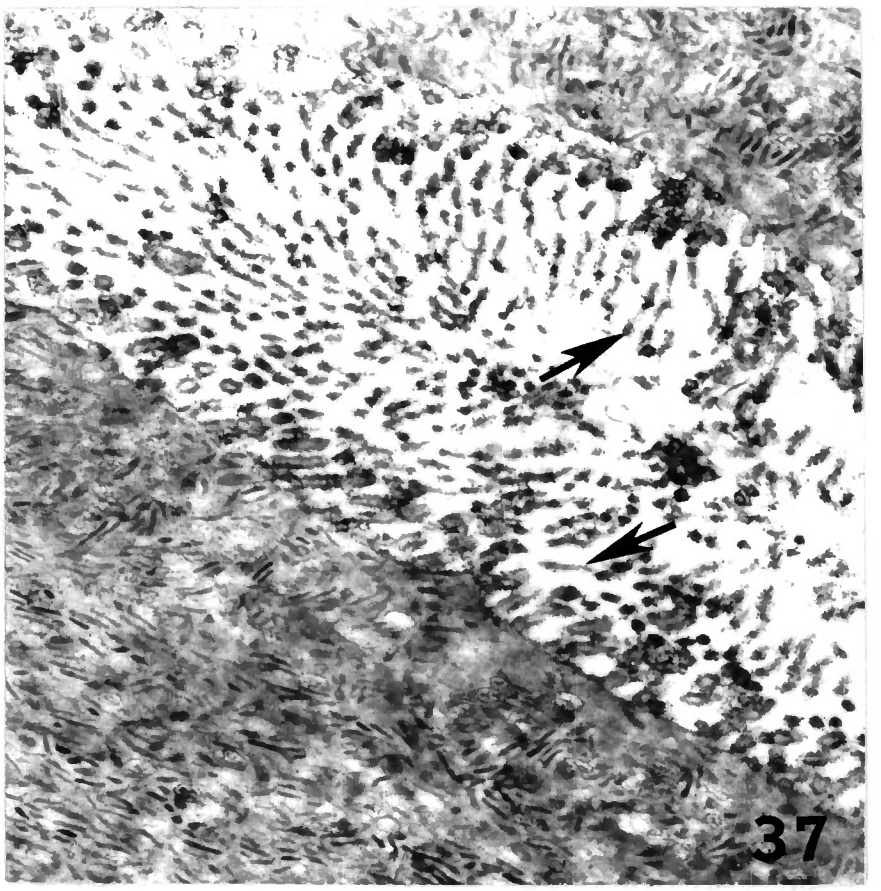

DC
PC
35

Fig. 36. Alkaline phosphatase activity on microthrix border (Mt) inhibited by 1 mM NaF. Note doughnut-shaped structure about 0.1 micron in diameter (arrow), unstained (x 24,000).

Fig. 37. Alkaline phosphatase activity on microthrix borders (arrows) partially inhibited by 10 mM EDTA, unstained (x 20,000).


Mt
36

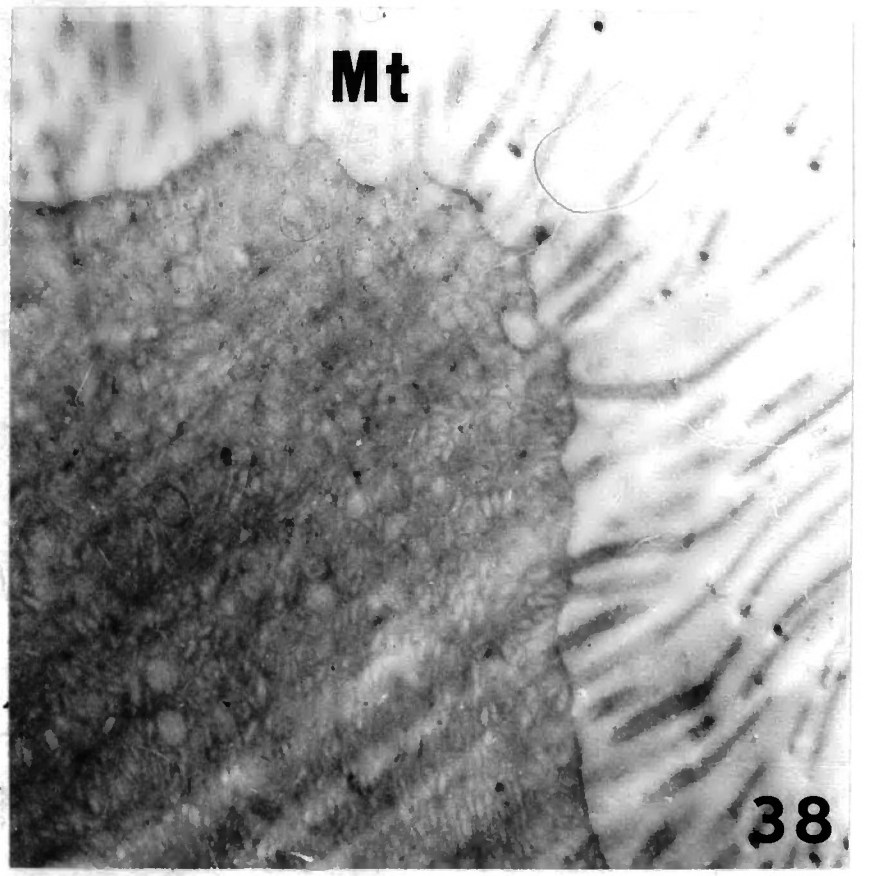

37

activity (Fig. 38). In transverse sections, alkaline phosphatase activity was observed around the cores of microtriches, in the cytoplasm of the perinuclear region, subtegumental cells and around muscle bundles. No activity was observed in the mitochondria (Figs. 39, 40). Tissues incubated in medium lacking the substrate showed an absence of enzyme activity (Figs. 41, 42). Alkaline phosphatase activity was observed in the cytoplasmic processes of cells, cytoplasm of subtegumental cells and longitudinal muscle bundles. No activity was seen in the nuclei of cells, smooth-surface vesicles and vesiculated cisternae of endoplasmic reticulum (Figs. 43, 44). Cytoplasmic processes of cells in control tissues showed an absence of enzyme activity (Fig. 45).

Alkaline phosphatase activity was not visualized in the flame cells of tissues but the reaction product was found around circular muscle bundles nearby (Fig. 46). Circular muscle bundles of control tissues showed no evidence of the reaction product (Fig. 47). The intercellular space of adjacent cells showed heavy deposits of alkaline phosphatase activity while control tisses from a similar region showed an absence of enzyme activity (Figs. 48, 49). An intense deposition of granular deposits was found in calcareous corpuscles of tissues incubated in a substrate-medium (Fig. 50). Control tissues of calcareous corpuscles without the substrate showed an absence of enzyme activity (Fig. 51).

Fig. 38. Substrate-contol, heated 1 min at 90 C prior to fixation. Note absence of alkaline phosphatase activity on microthrix border (Mt), unstained (x 20,000).

Fig. 39. Transverse section showing alkaline phosphatase activity around the cores of microtriches (CM) and in the cytoplasm (arrow), unstained (x 19,200). Subtegumental cell (SC); Mitochondria (M).


Mt
38

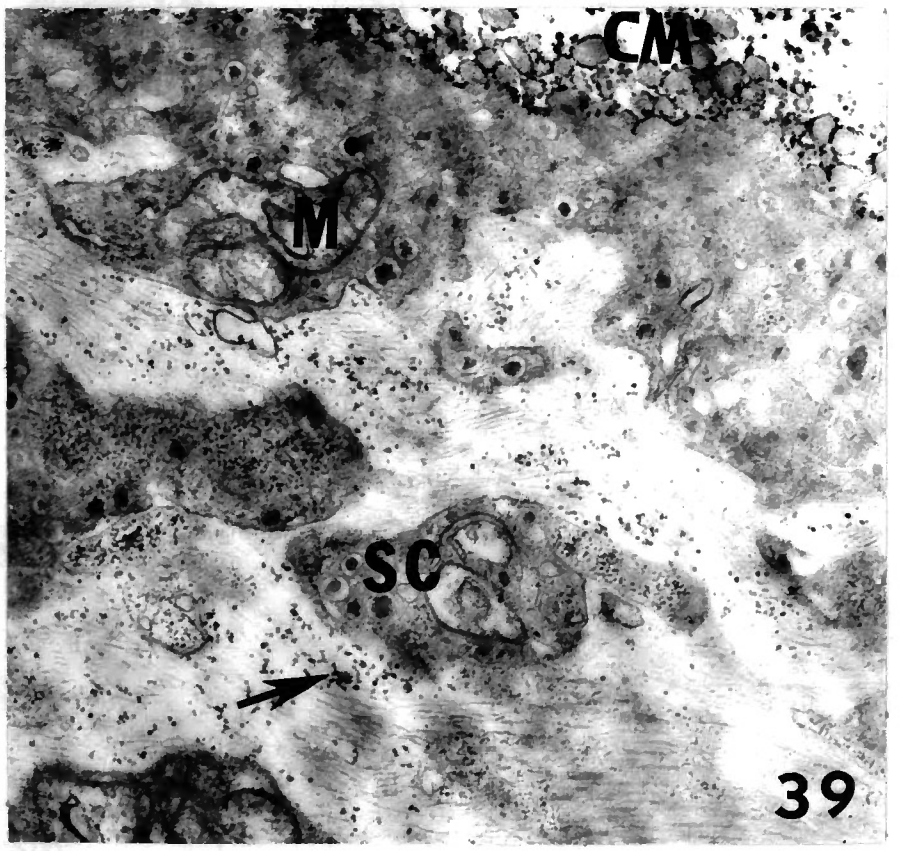
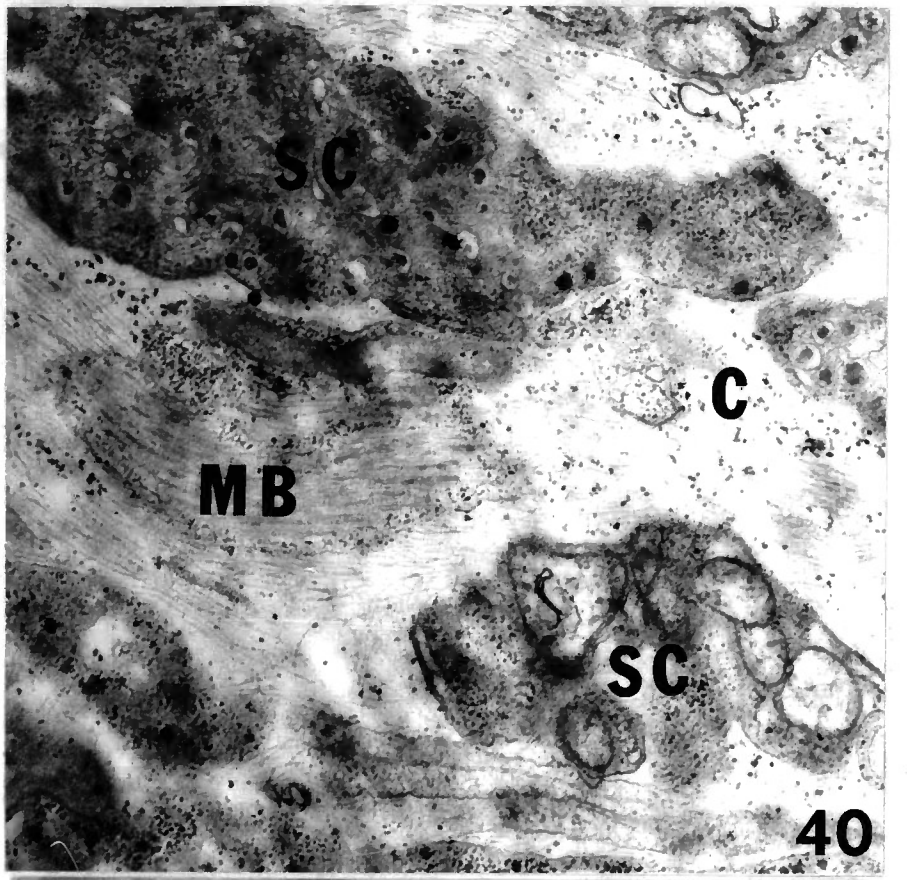

CM
M
SC
39

Fig. 40. Transverse section showing alkaline phosphatase activity in cytoplasm (C), subtegumental cells (SC) and around muscle bundles (MB), unstained (x 19,200).

Fig. 41. No-substrate control, showing absence of alkaline phosphatase activity in subtegumental cell (SC), unstained (x 30,000). Smooth-surface vesicle (arrow); Nucleus (N).


SC
C
MB
SC
40

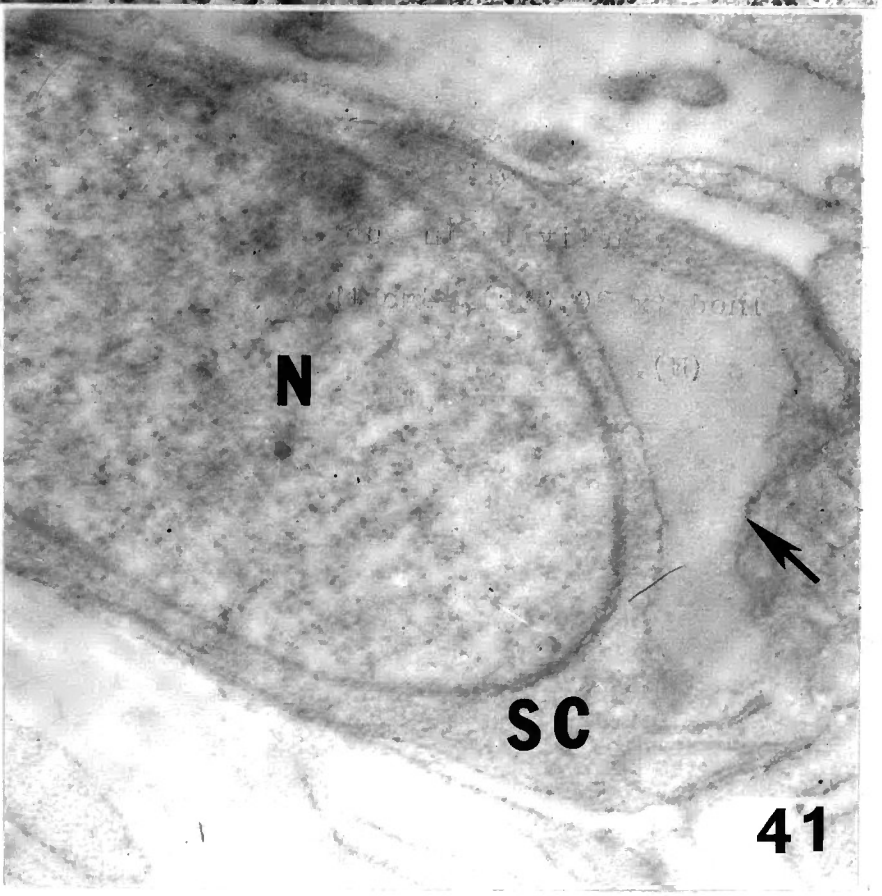
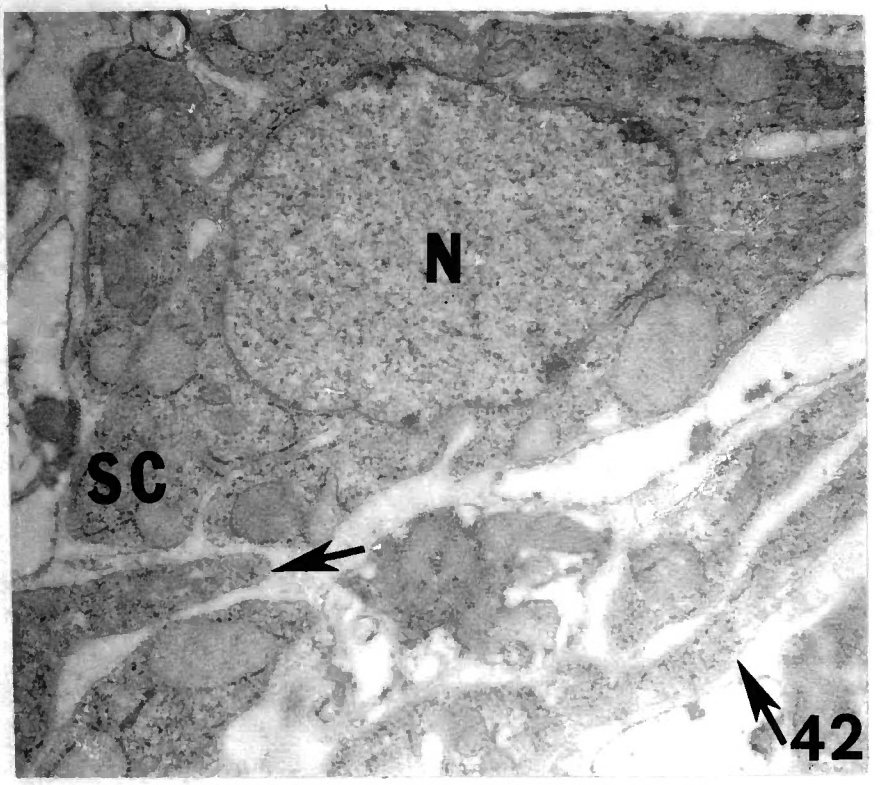

N
SC
41

Fig. 42. No-substrate control, showing absence of alkaline phosphatase activity in subtegumental cell (SC) and cytoplasmic processes of cells (arrows), unstained (x 20,000). Nucleus (N).

Fig. 43. Alkaline phosphatase activity in cytoplasmic processes of cells (arrow), cytoplasm of subtegumental cell (SC), unstained (x 24,000). Smooth-surfaced vesicle (SSV); Vesiculated cisternae of granular endoplasmic reticulum (VER).


N
SC
42

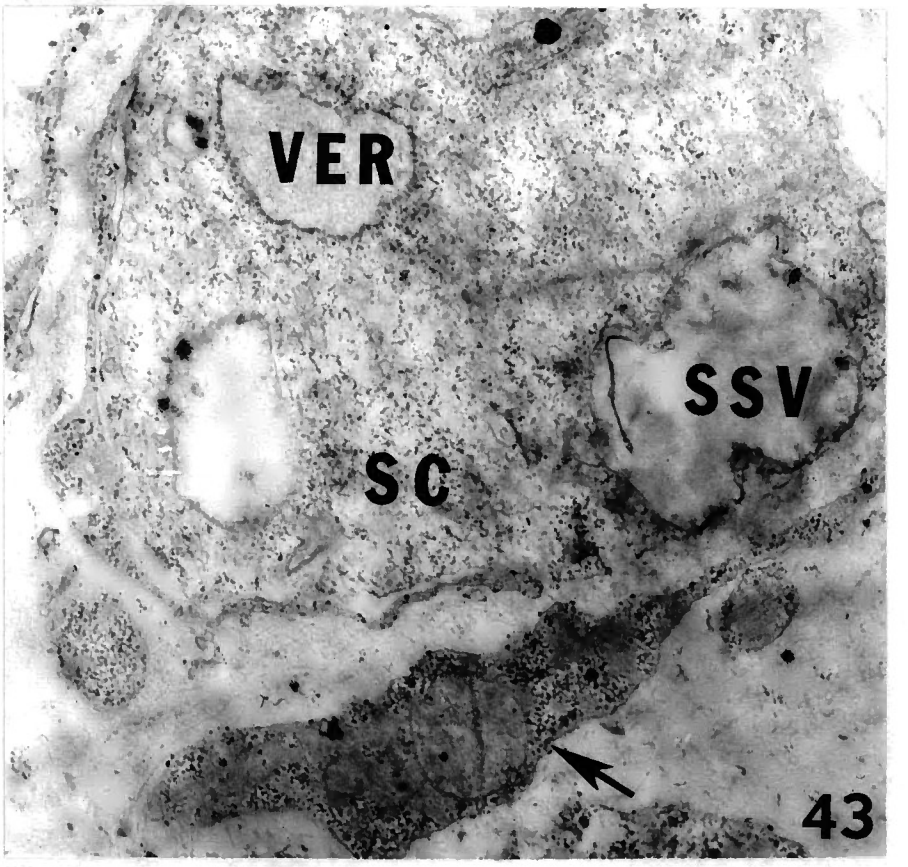
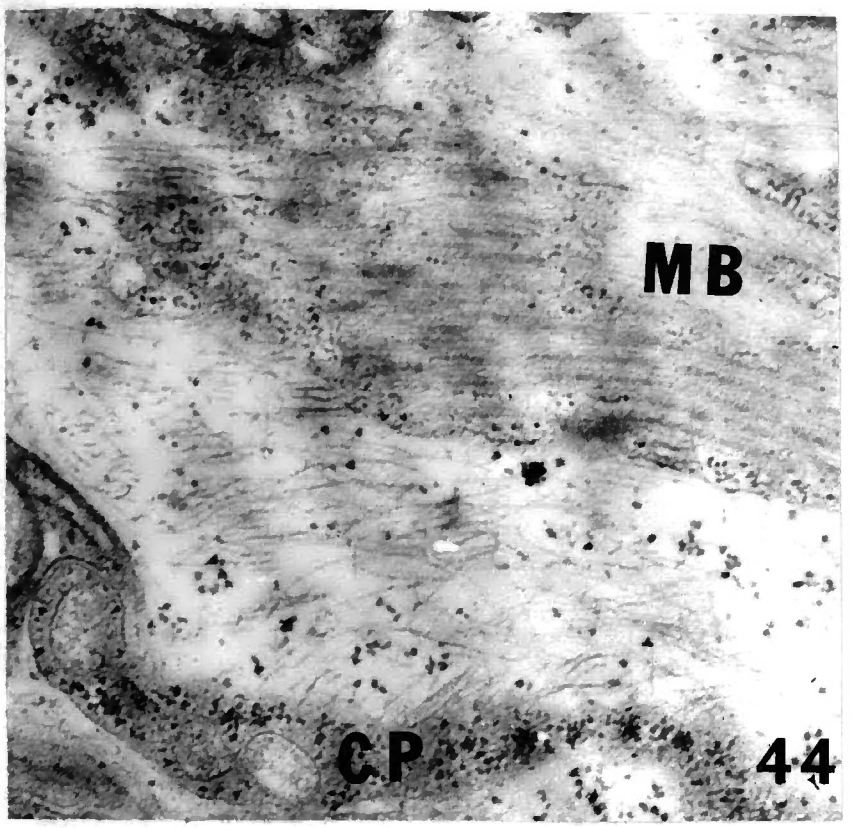

VER
SSV
SC
43

Fig. 44. Alkaline phosphatase activity around a longitudinal muscle bundle (MB) and portions of a cytoplasmic process of a cell (CP), unstained (x 18,000).

Fig. 45. No-substrate control, showing absence of alkaline phosphatase activity in portions of a cytoplasmic process of a cell (CP, subtegumental cell (SC) and cytoplasm (C), unstained (x 24,800). Vacuole (Vac).


MB
CP
44

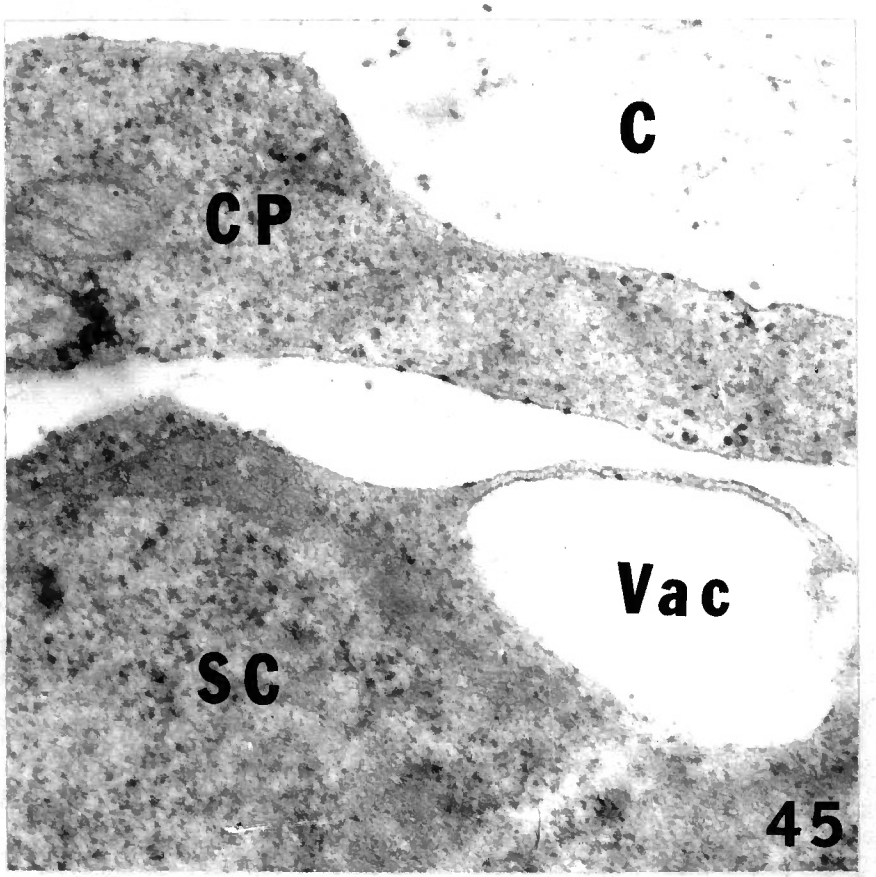
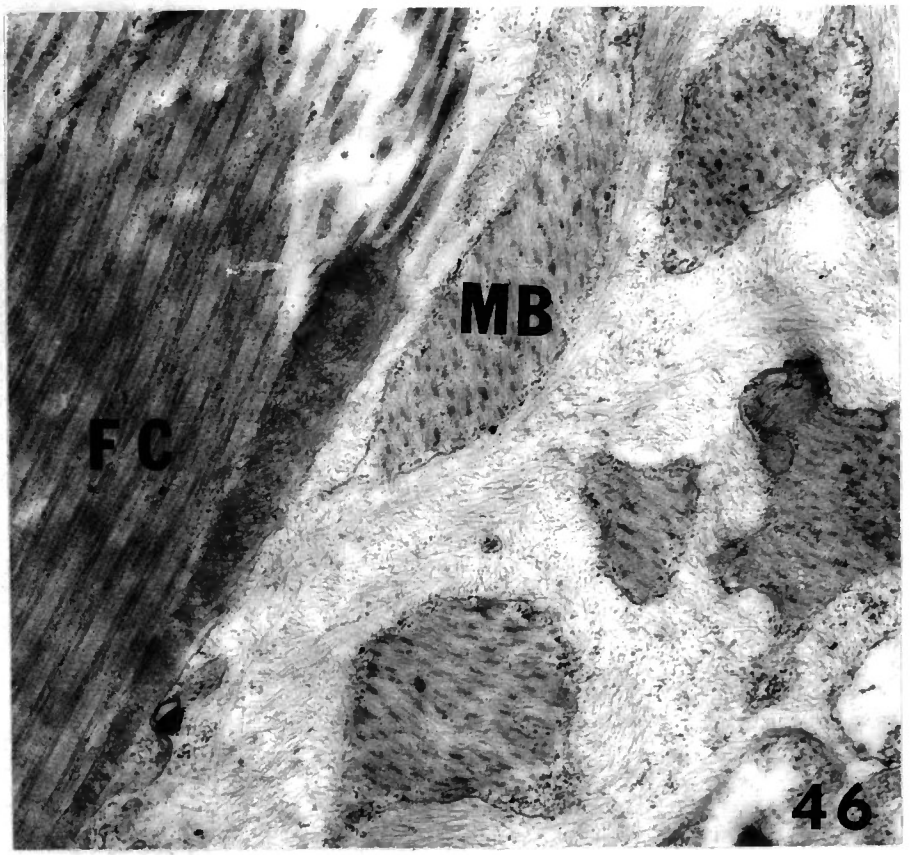

C
CP
Vac
SC
45

Fig. 46. Alkaline phosphatase activity around circular muscle bundles (MB), unstained (x 20,400). Flame cell (FC).

Fig. 47. No-substrate control, showing absence of alkaline phosphatase activity around muscle bundle (MB) and in subtegumental cell (SC), unstained (x 20,000). Nucleus (N); Mitochondria (M).


MB
FC
46

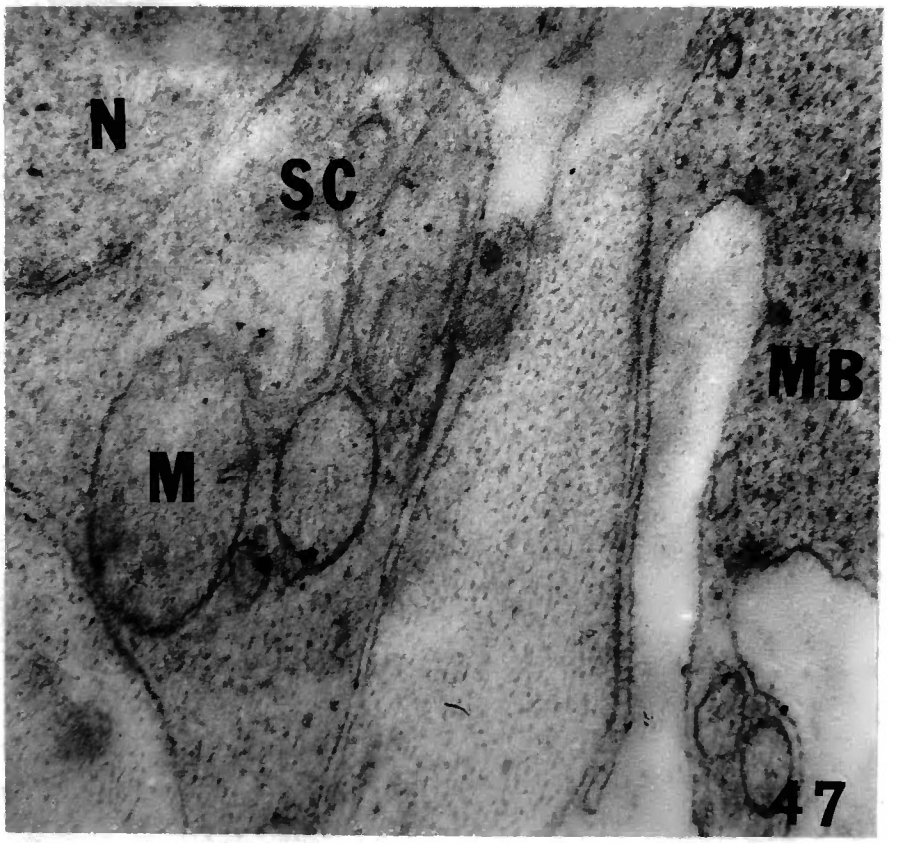
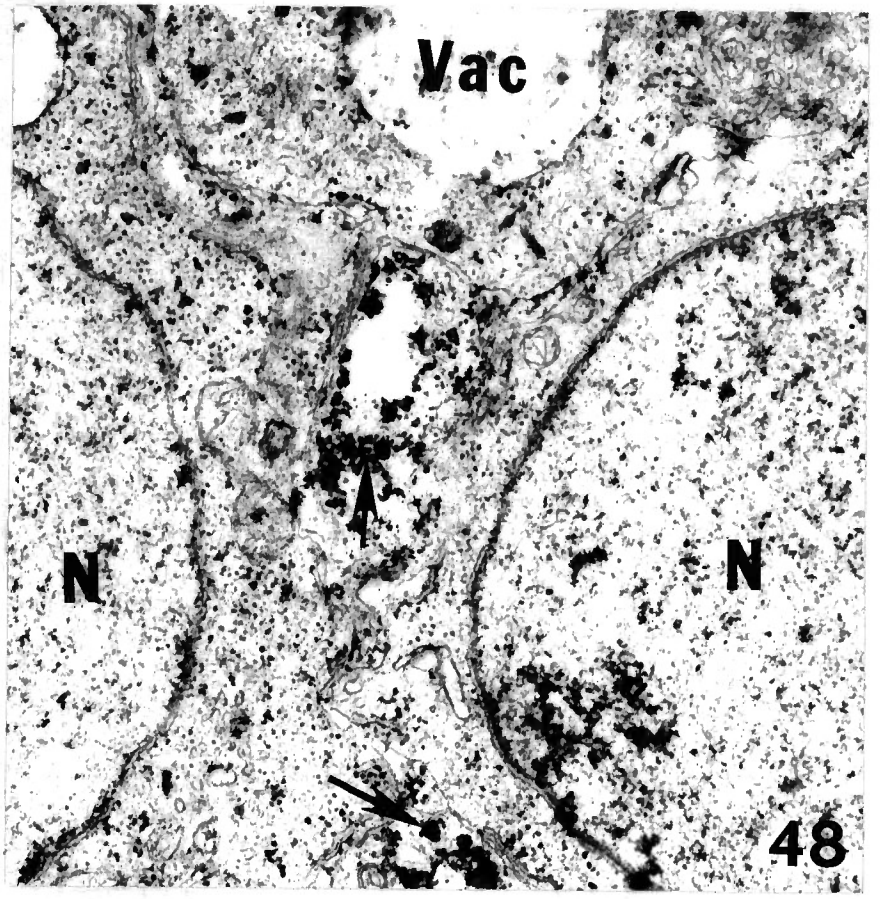

N
SC
MB
M
47

Fig. 48. Alkaline phosphatase activity in intercellular space of adjacent cells (arrows), unstained (x 20,000). Nuclei (N); Vacuole (Vac).

Fig. 49. No-substrate control, showing absence of alkaline phosphatase activity in intercellular space of adjacent cells (arrows), unstained (x 20,000). Nuclei (N).


Vac
N
N
48

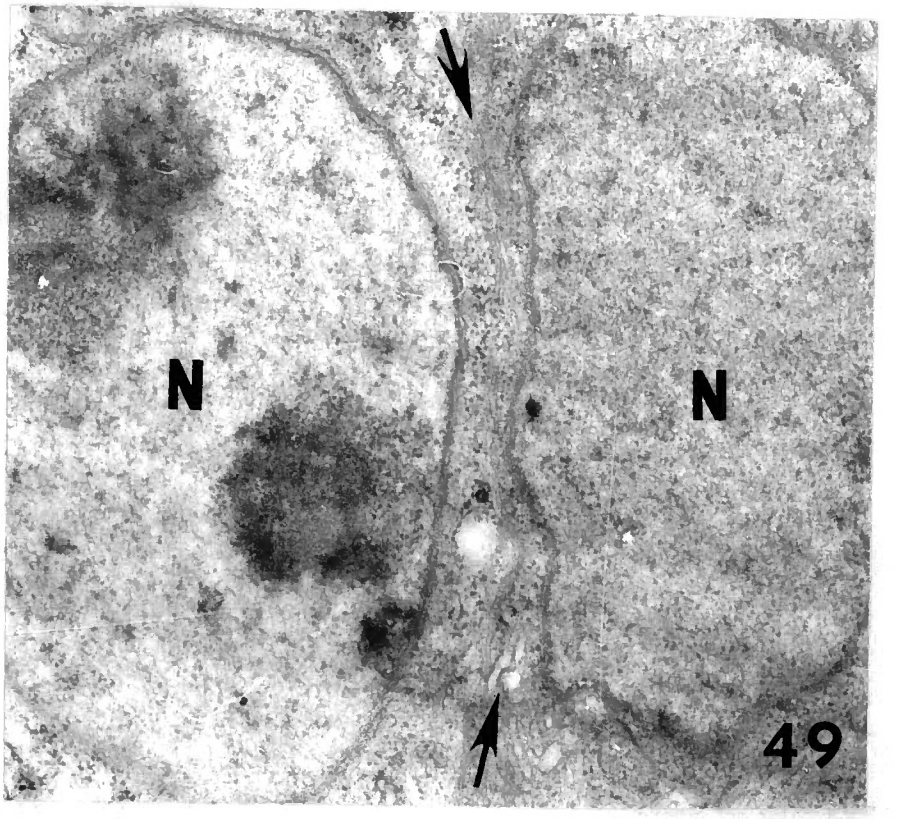
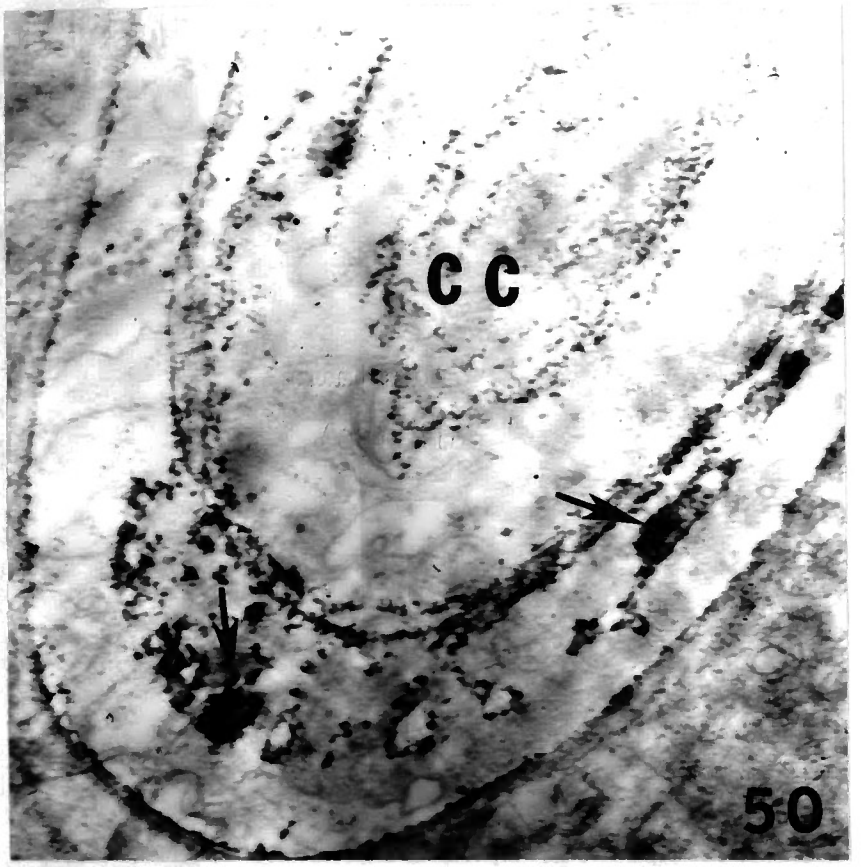
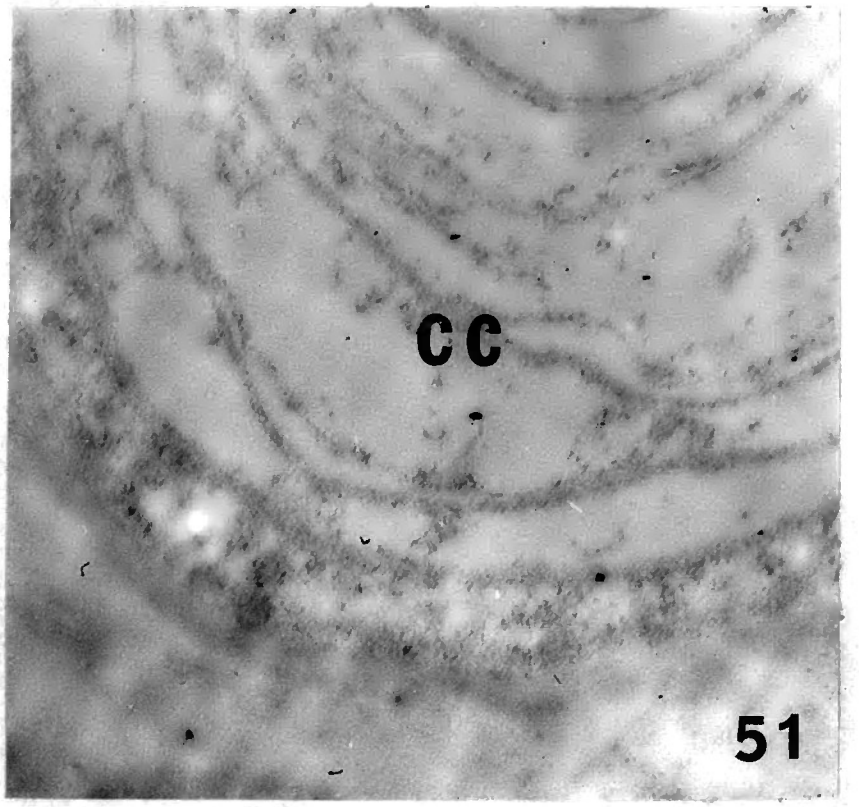

N
N
49

Fig. 50. Alkaline phosphatase activity in a calcareous corpuscle (arrows), unstained (x 30,000). Calcareous corpuscle (CC).

Fig. 51. No-substrate control, showing absence of alkaline phosphatase activity in a calcareous corpuscle (CC), unstained (x 30,000).


CC
50


CC
51

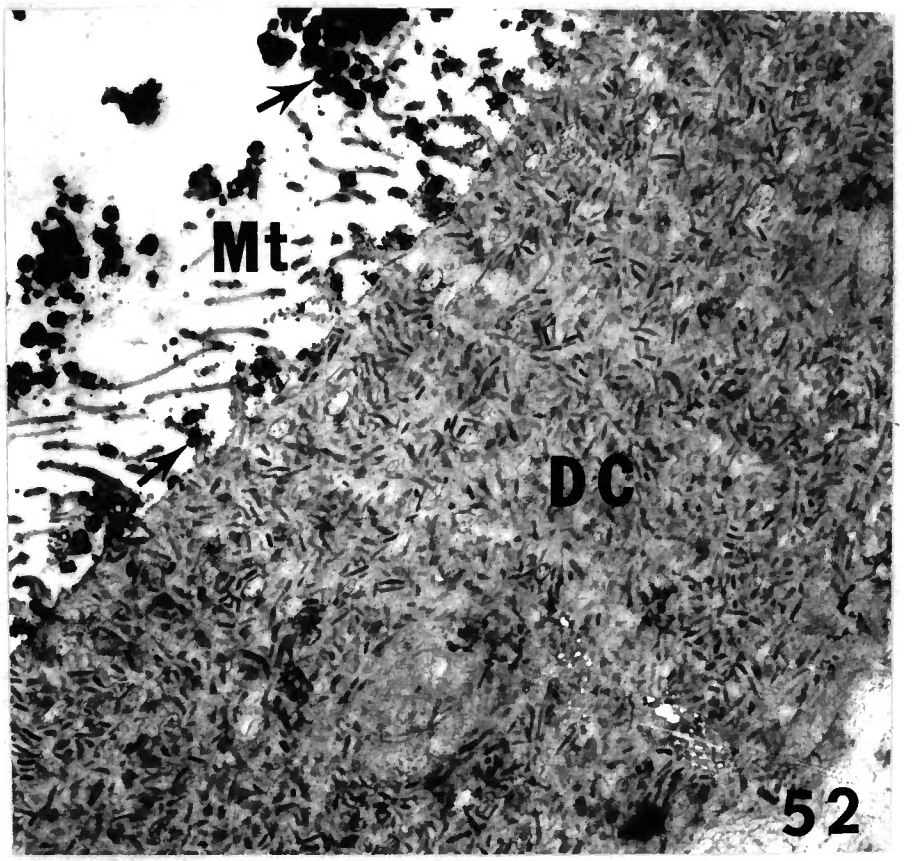
Ultrastructural Localization of Acetylcholinesterase Activity in the Scolex

The ultrastructural localization of acetylcholinesterase activity was demonstrated in the scolex of the cysticercus of H. taeniaeformis. Electron dense deposits were taken as an indication of enzyme activity. The reaction product was observed on the microtriches of the distal cytoplasm (Figs. 52, 53). In the absence of the substrate, no reaction product was seen (Fig. 54). An intense reaction was seen in a muscle and surrounding cytoplasm of the rostellar region (Fig. 55). Control tissues in the absence of the substrate was negative for enzyme activity (Fig. 56). Acetylcholinesterase activity was also observed in processes of a muscle bundle (Fig. 57). The use of 10^{-4} M eserine inhibited enzyme activity in the muscles (Fig. 58). Enzyme activity was inhibited in all sections examined using 10^{-4} M eserine. The reaction product was observed in large vesiculated bodies (Fig. 50). Muscle bundles heated for 1 min prior to fixation at 90 C and subsequently placed in a substrate-medium showed an absence of acetylcholinesterase activity (Fig. 60).

Acetylcholinesterase activity was also observed in nerve cells. Activity was localized within the nuclear envelope, in the cytoplasm and membrane of a nerve cell in the sucker region (Fig. 61). Control tissues in the absence of a substrate

Fig. 52. Acetylcholinesterase activity on the microtriches (Mt) (arrows) of the scolex, unstained (x 20,000). Distal cytoplasm (DC).

Fig. 53. Higher magnification of microtriches showing acetylcholinesterase activity (arrows), unstained (54,400). Distal cytoplasm (DC).


Mt
DC
52

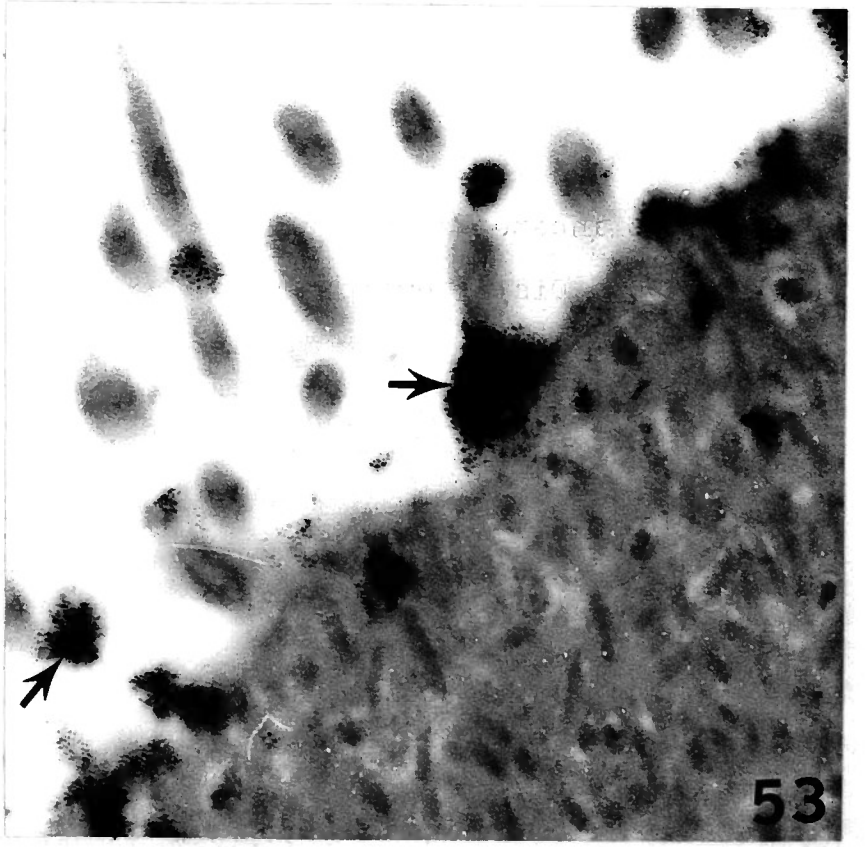
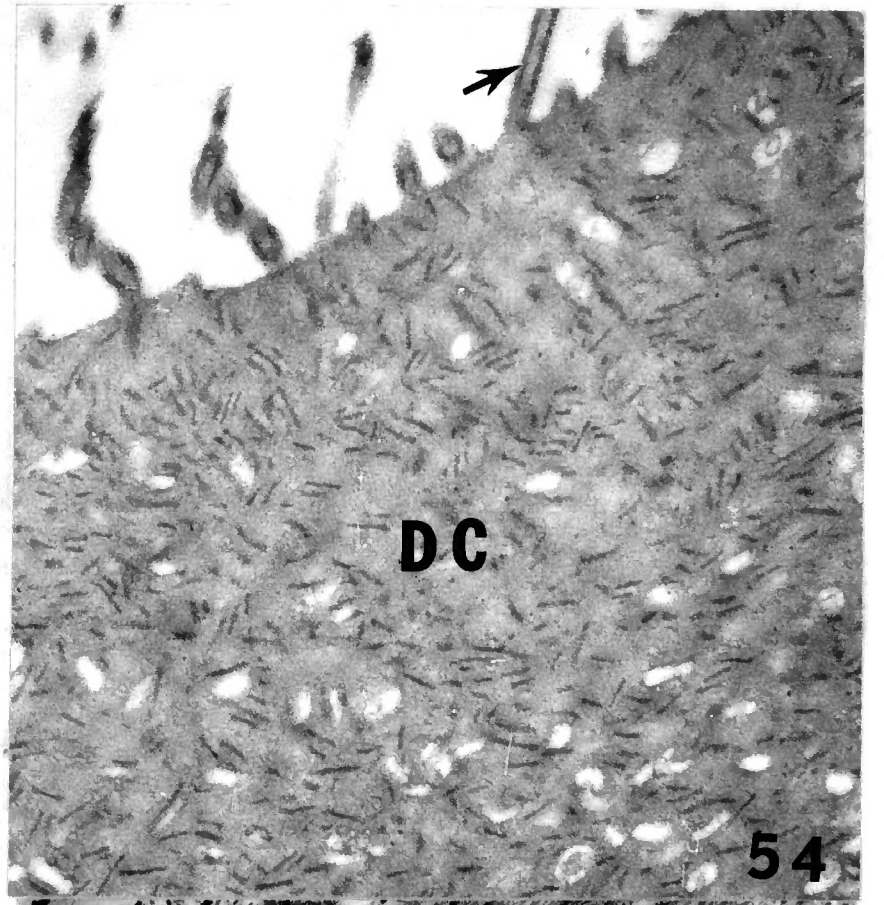

53

Fig. 54. No-substrate control. Note absence of acetylcholinesterase activity on the microtriches (arrow), unstained (x 20,000). Distal cytoplasm (DC).

Fig. 55. Longitudinal section through the rostellum showing acetylcholinesterase activity in a muslce bundle (MB) and cytoplasm (C), unstained (x 20,000).


DC
54

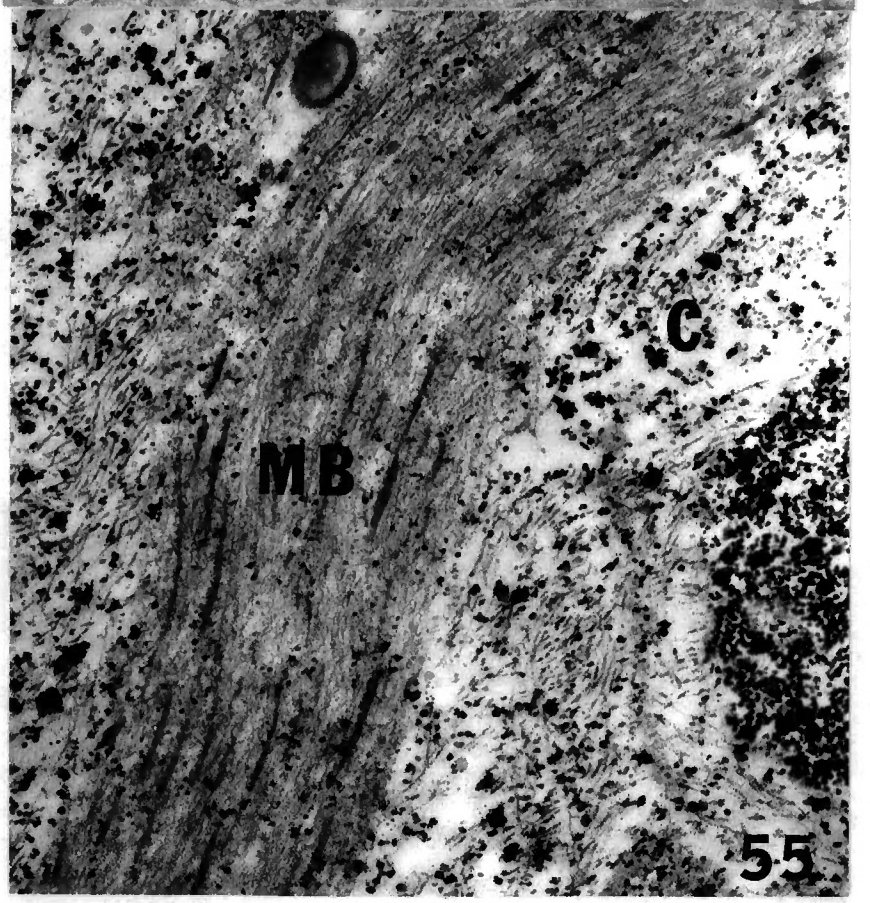
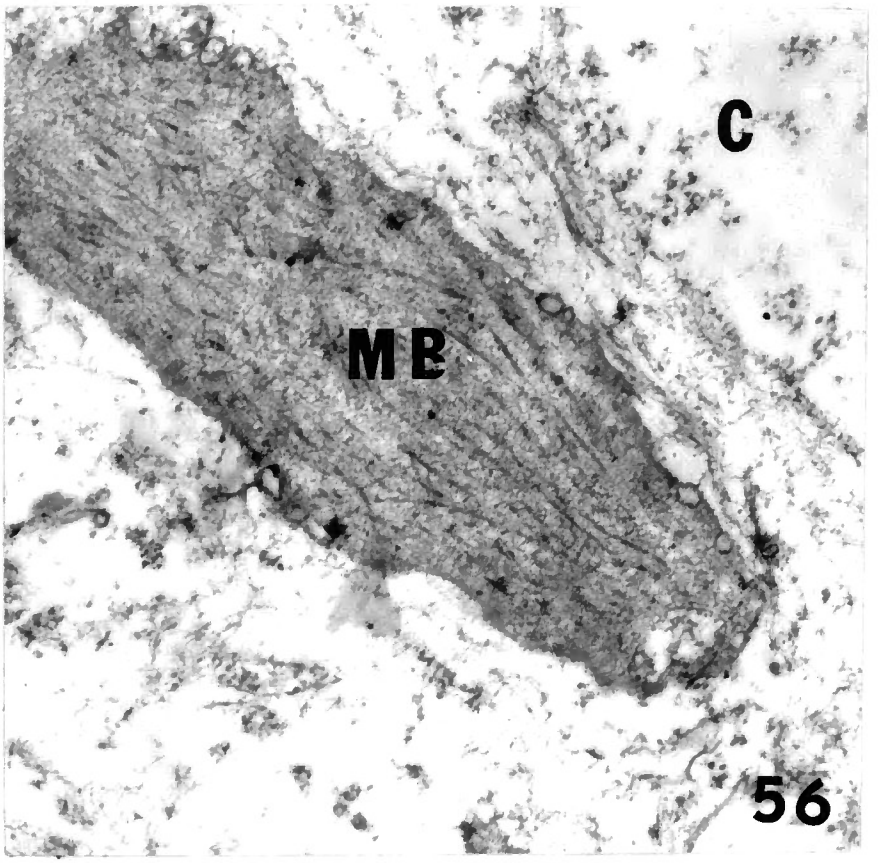

C
MB
55

Fig. 56. No-substrate control showing absence of acetylcholinesterase activity in a muscle bundle (MB) and surrounding cytoplasm (C), unstained (x 20,000).

Fig. 57. Acetylcholinesterase activity in processes of a muscle bundle (MB) and surrounding cytoplasm (C), unstained (x 20,000).


C
MB
56

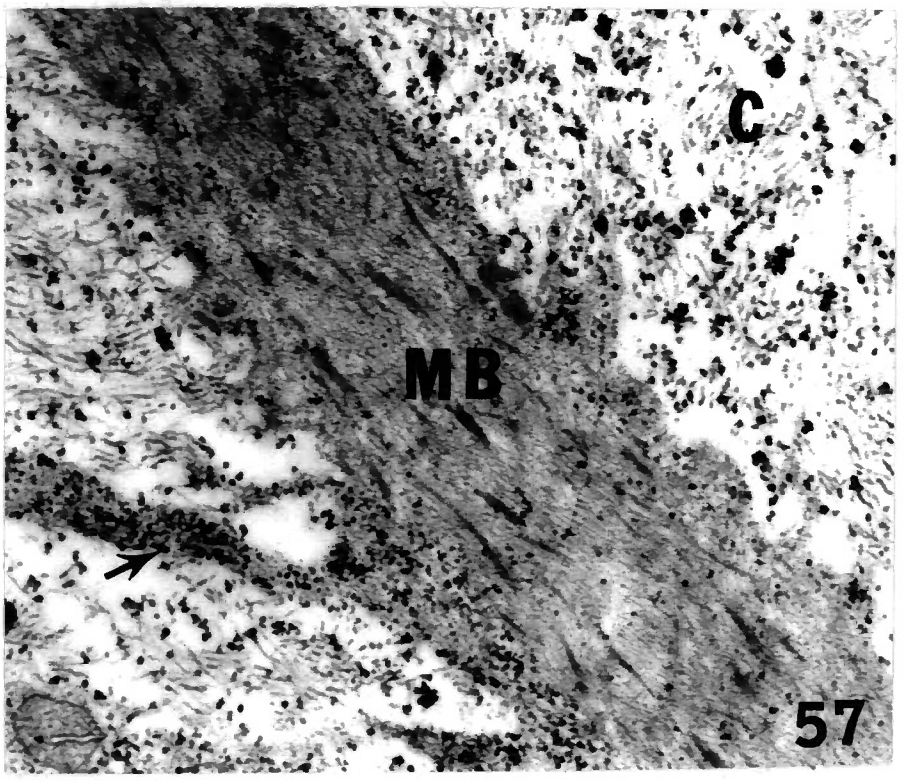
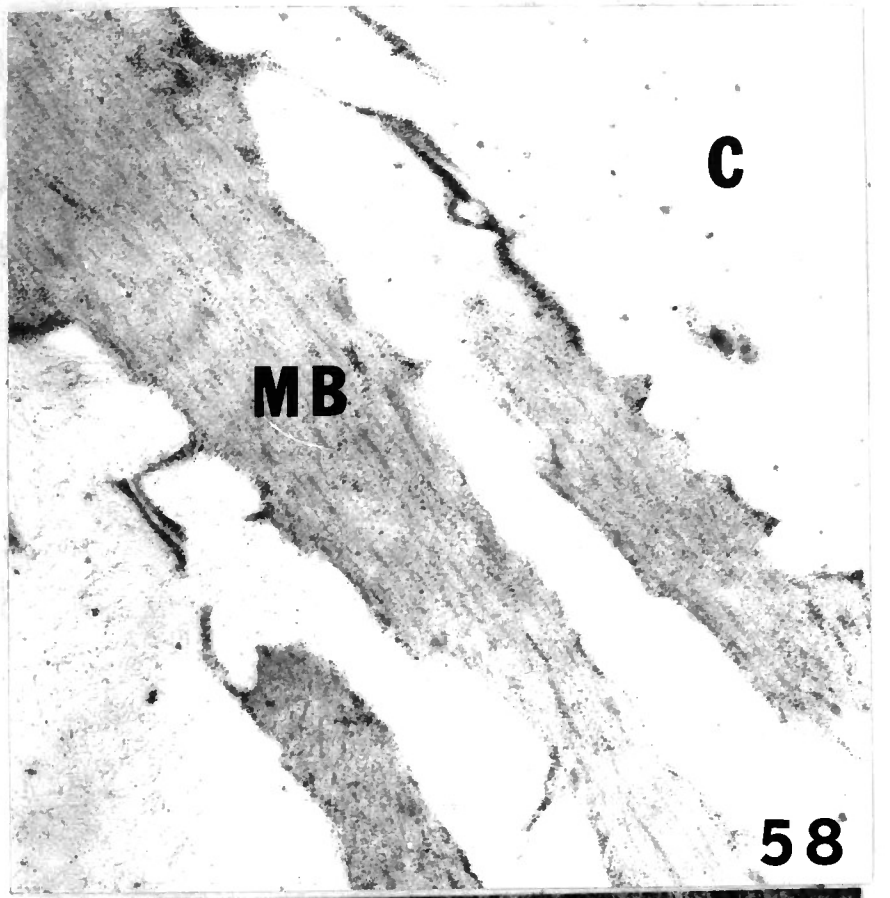

C
MB
57

Fig. 58. Acetylcholinesterase activity in a muscle bundle (MB) and surrounding cytoplasm (C) inhibited by 10^{-4} M eserine, unstained (x 20,000).

Fig. 59. Acetylcholinesterase activity in a large vesiculated (VB) and muscle bundle (MB), unstained (x 20,000). Reticulin fibers (arrows).


C
MB
58

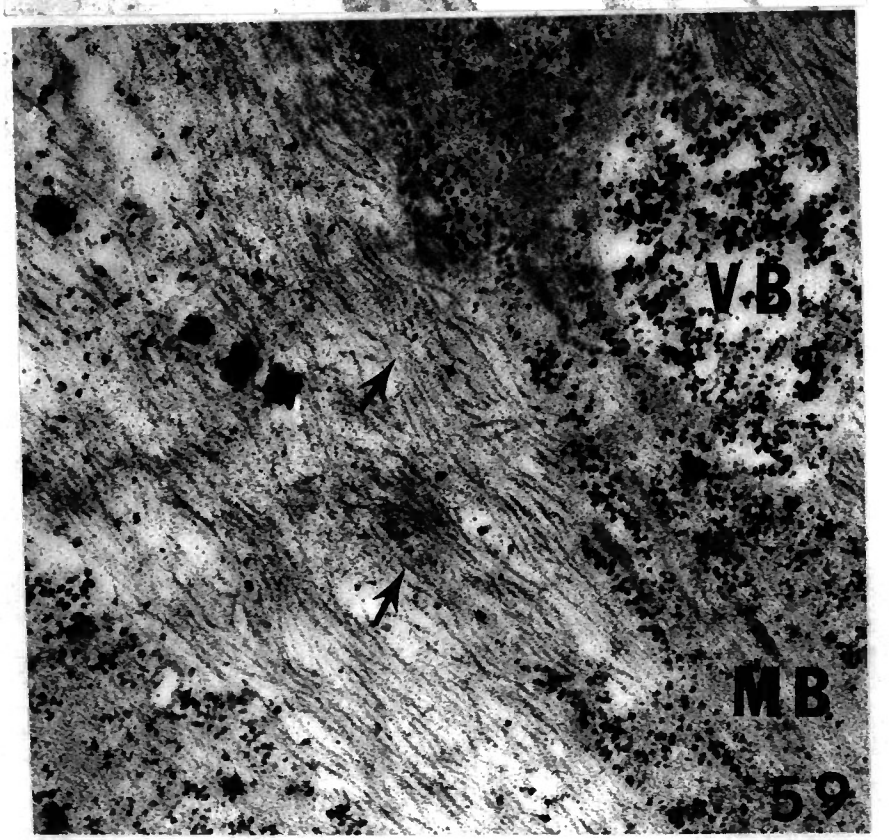
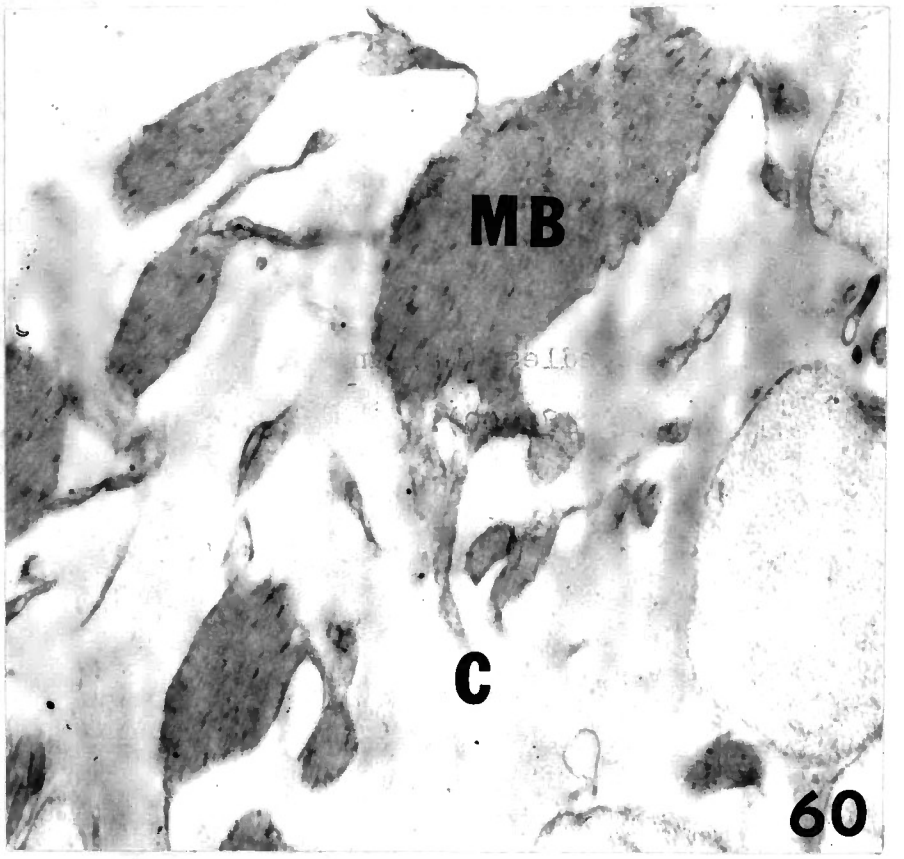
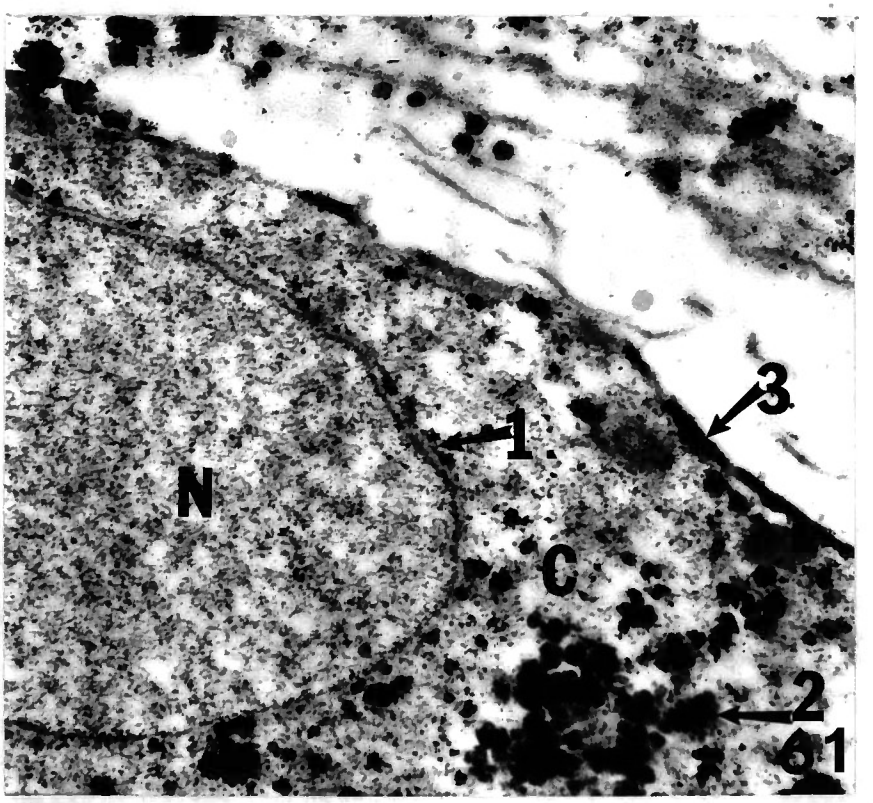

VB
MB
59

Fig. 60. Substrate-control heated 1 min prior to fixation at 90 C. Note absence of acetylcholinesterase activity in muscle bundles (MB) and surrounding cytoplasm (C), unstained (x 18,000).

Fig. 61. Acetylcholinesterase activity within the nuclear envelope (arrow[1]), cytoplasm (C) (arrow[2]) and membrane of a nerve cell (arrow[3]) in the region of the suckers, unstained (x 26,400) Nucleus (N).


MB
C
60

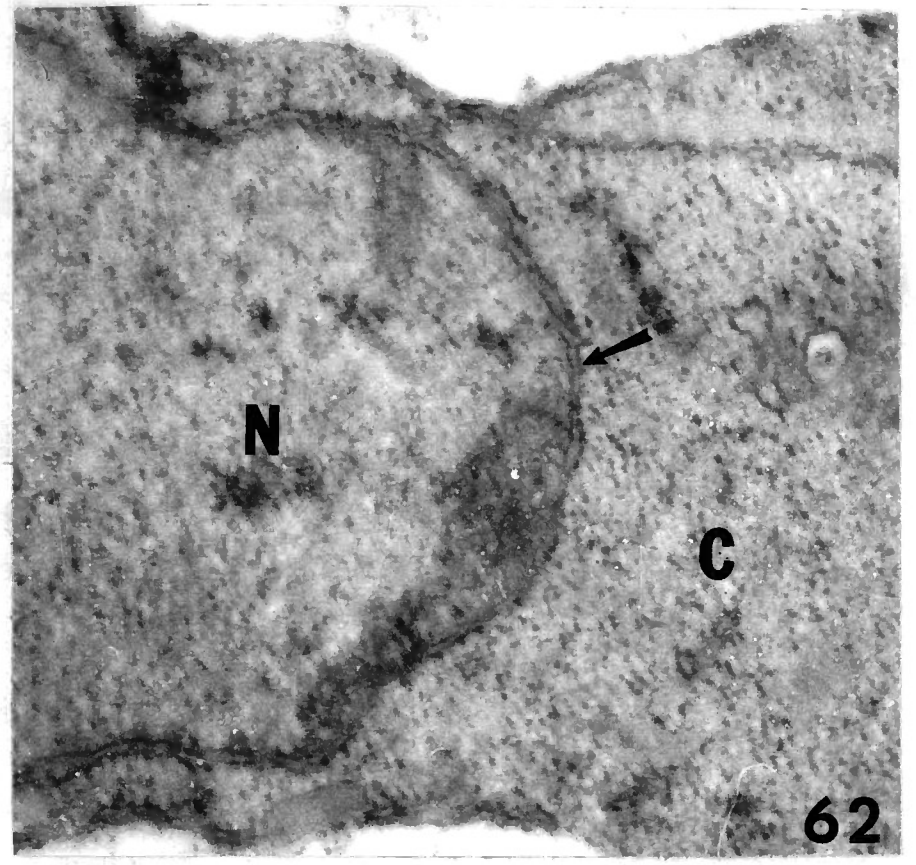

3
1
N
C
2
61

showed an absence of enzyme activity within the nuclear envelope, cytoplasm and membrane of a nerve cell (Fig. 62). Acetylcholinesterase activity was also observed in nerve cells in the rostellar region. Activity was found within the nuclear envelope, cytoplasm and membrane of nerve cells (Fig. 63). Enzyme activity was abolished in nerve cells when 10^{4} M eserine was placed in the substrate-medium (Fig. 64).

The reaction product was demonstrated around the membrane of cilia in flame cells (Fig. 65). Flame cells in the absence of the substrate showed no acetylcholinesterase activity (Fig. 66). Enzyme activity was inhibited with 10^{-4} M eserine (Fig. 67). A sagittal section of a flame cell also showed the reaction product (Fig. 68). A high degree of activity was observed in the hooks of the rostellum (Fig. 69). Hooks of control tissues in a substrate-free medium did not show the reaction product (Fig. 70).

Fig. 62. No-substrate control showing absence of acetylcholinesterase activity within the nuclear envelope (arrow) and cytoplasm (C), unstained (x 26,400). Nucleus (N).

Fig. 63. Acetylcholinesterase activity inhibited with 10^{-4} M eserine within the nuclear envelope (arrow) of a nerve cell, unstained (x 30,000). Nucleus (N); electron dense bodies (DB); Vesicles (Ves).


N
C
62

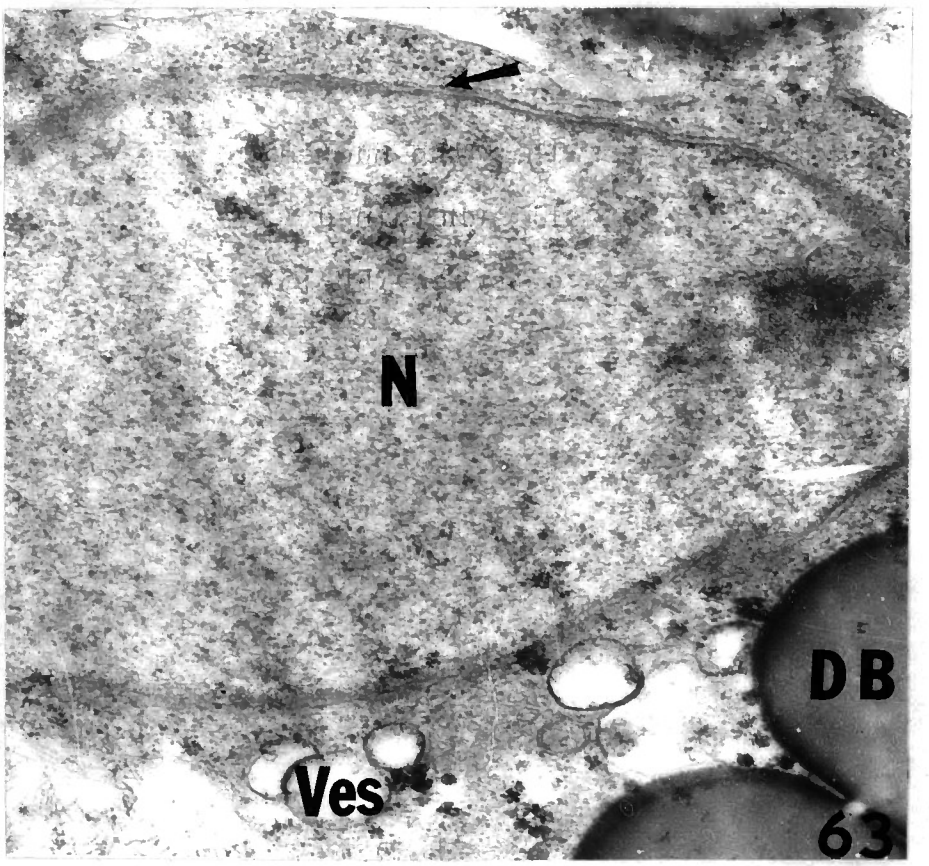
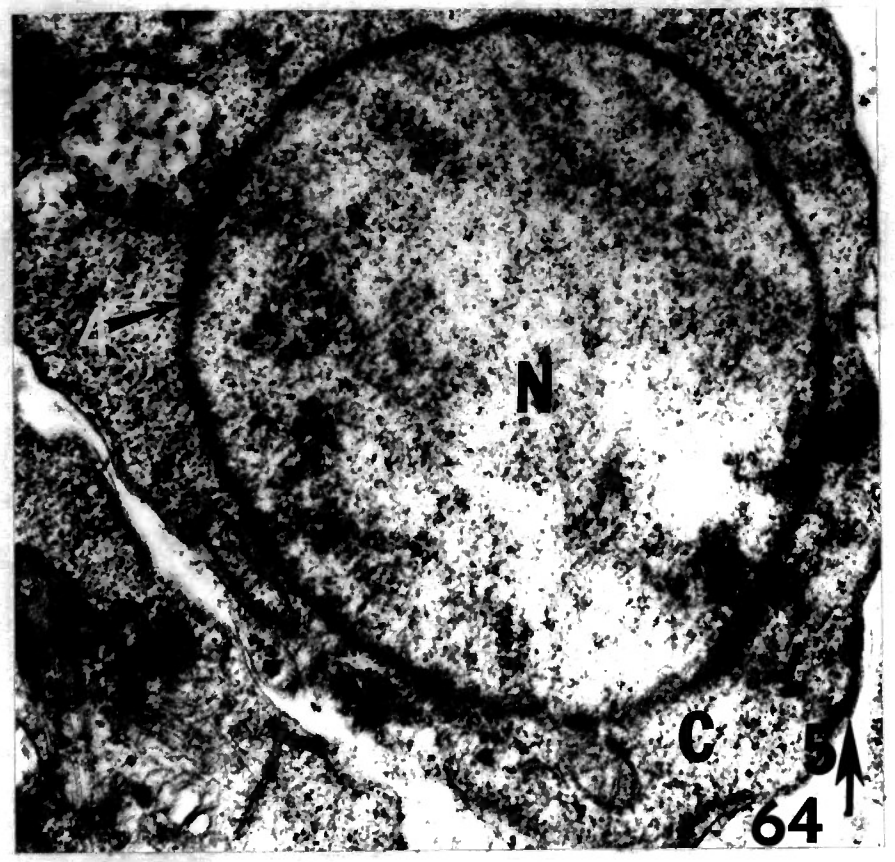

N
DB
Ves
63

Fig. 64. Acetylcholinesterase activity within the nuclear envelope (arrow[4]) and in the cytoplasm (C) and membrane of a nerve cell (arrow[5]) in the rostellar region (x 26,400).

Fig. 65. Acetylcholinesterase activity around the membranes of cilia (arrows) in a flame cell of the scolex, unstained (x 24,000).


N
C
64

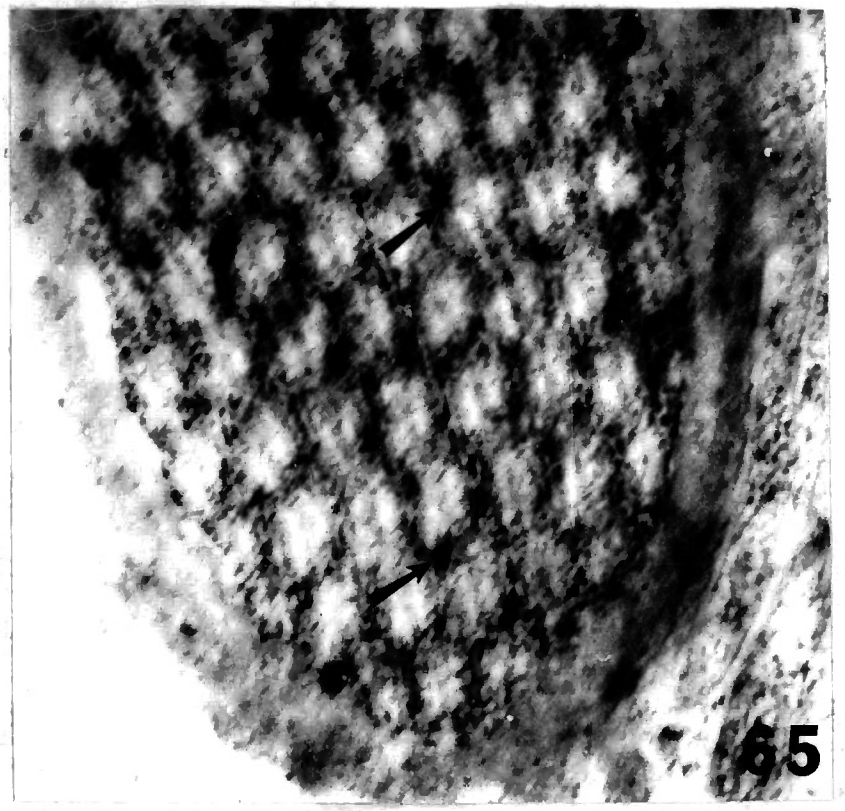
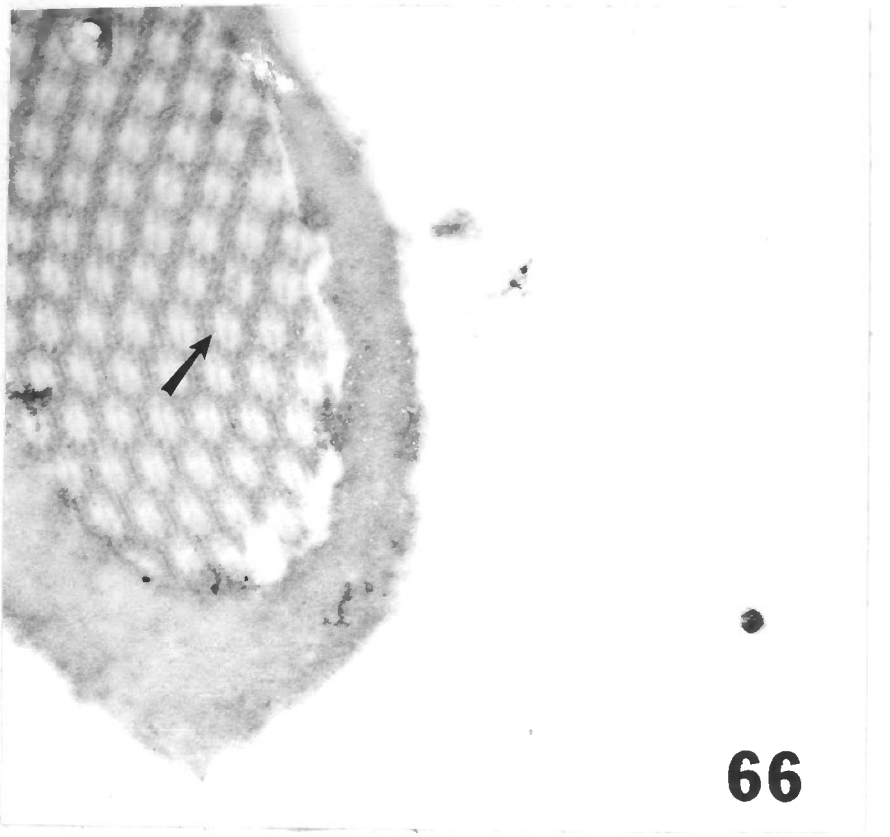
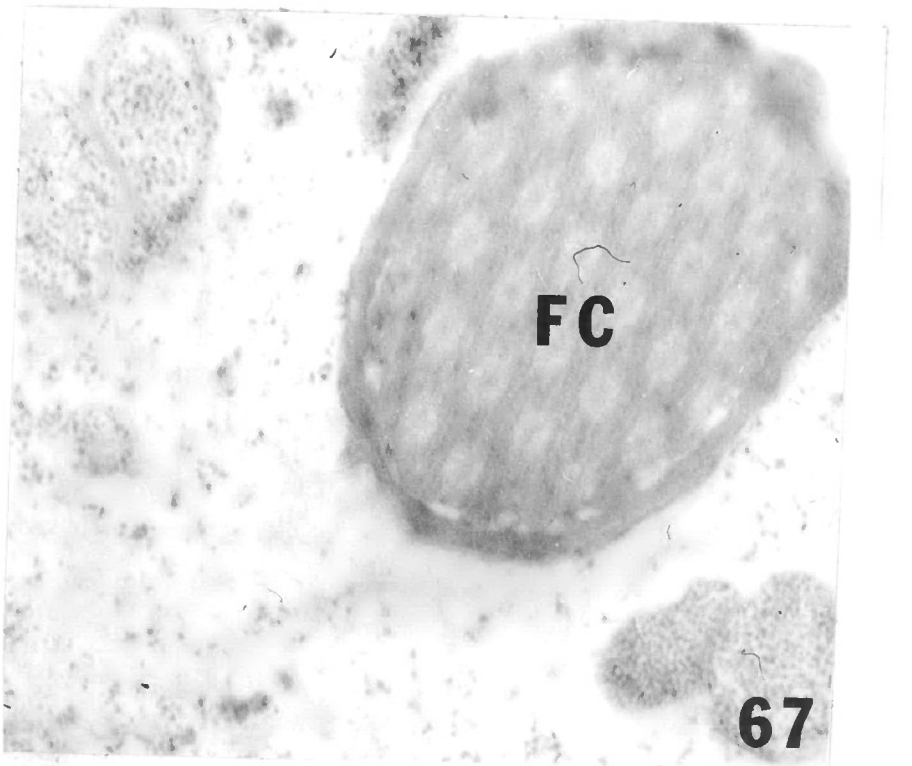

65

Fig. 66. No-substrate control showing absence of acetylcholinesterase activity around the membranes of cilia (arrow) in a flame cell, unstained (x 24,000).

Fig. 67. Acetylcholinesterase activity in a flame cell (FC) inhibited by 10^{-4} M eserine, unstained (x 18,000).



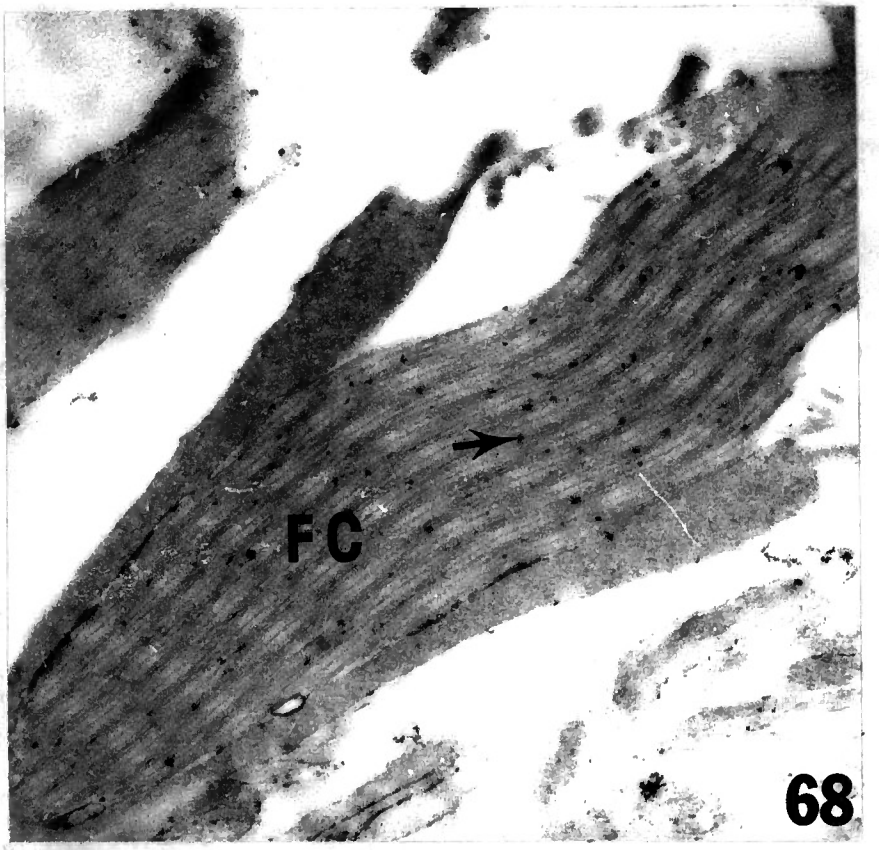
66



67

Fig. 68. Acetylcholinesterase activity in a sagittal section of a flame cell (FC) (arrow), unstained (x 40,000).

Fig. 69. Acetylcholinesterase activity in a section of a hook in the rostellum (arrows) of the scolex, unstained (x 36,000).


FC
68

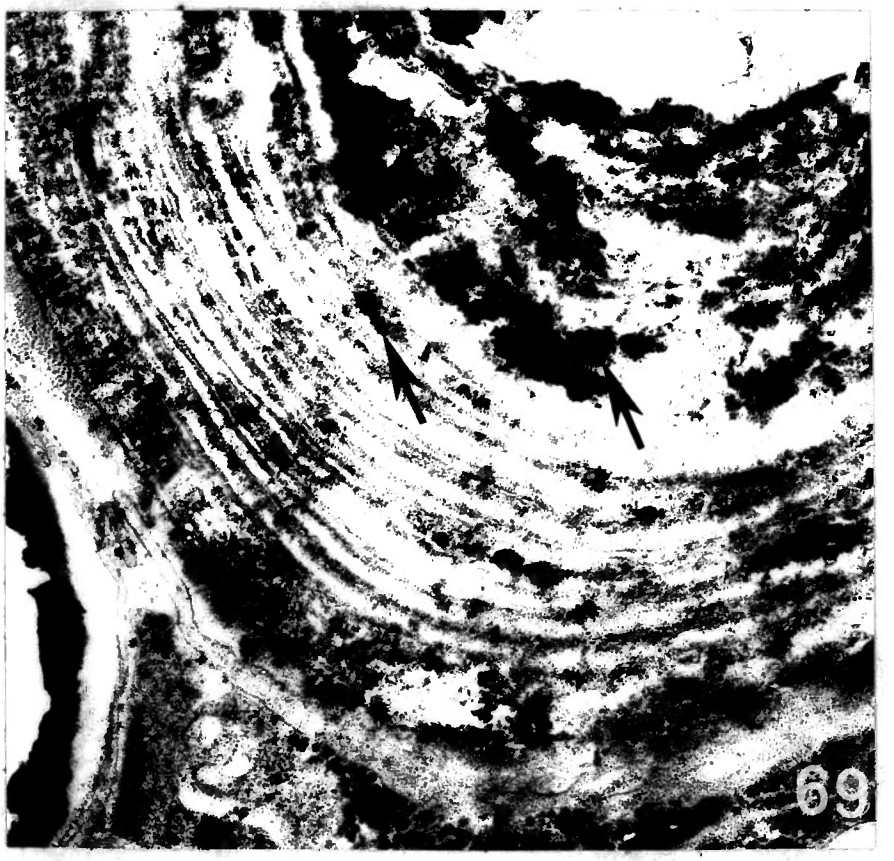
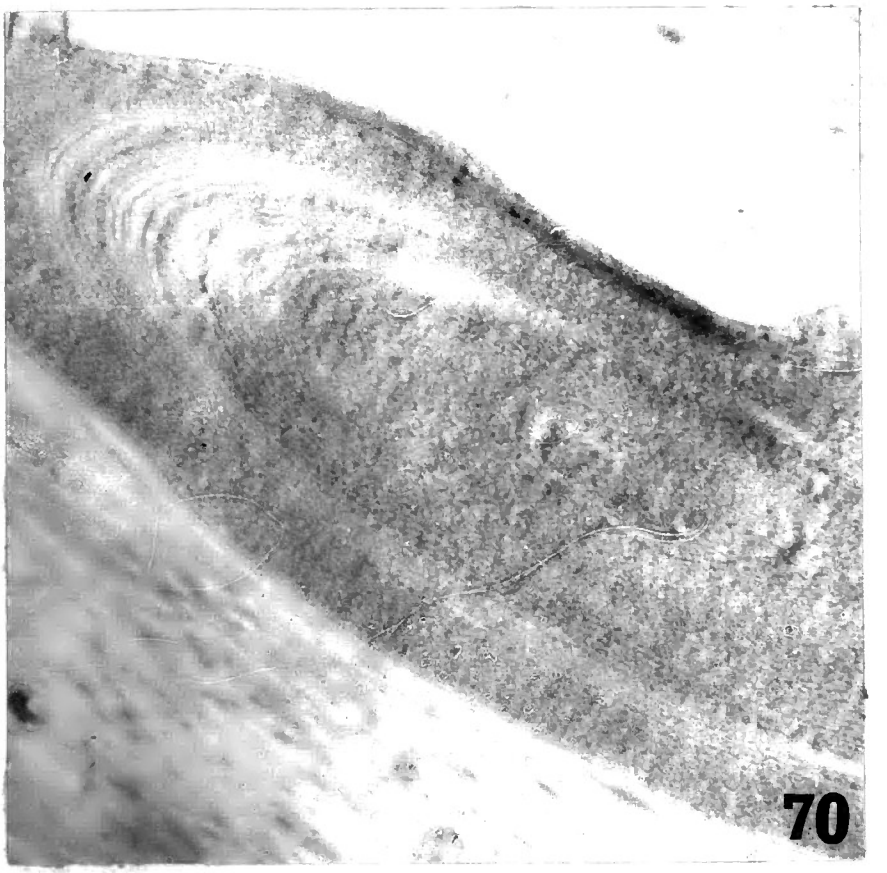

69

Fig. 70. No-substrate control showing absence of acetylcholinesterase activity in a hook of the rostellum, unstained (x 35,000).


70

CHAPTER V

DISCUSSION AND CONCLUSIONS

Macroscopic, SEM and ultracytochemical TEM studies have provided a better understanding of the morphology and chemical nature of the cysticercus of H. taeniaeformis.

SEM studies of the scolex of the cysticercus of H. taeniaeformis have revealed certain basic features such as ridges, depressions, cavities and folds. The presence of these features within the acetabula may aid in the attachment of the adult to the intestine of the host. Of special interest was the presence of microtriches within the cavities of the suckers and throughout the entire surface of the scolex. This is essentially in accord with reports published on several species of adult cestodes by Rees (1958) using light microscopy; by several investigators (Rosario, 1962; Rothman, 1963; Threadgold, 1962) using electron microscopy, and on other cestode larvae (Morseth, 1966, 1967a; Baron, 1968; Oaks and Lumsden, 1971; Blitz and Smith, 1973). Ubelaker et al.(1973) reported dense populations of microtriches on the rostellum, scolex and suckers of the cestode, H. diminuta. Berger and Mettrick (1971) studied the surface topography of H. diminuta by SEM and reported dimorphism in the microtriches of the mature and early gravid strobilar region. There was no mention of dimorphism in the microtriches of the scolex of H. diminuta. The microtriches of the cysticercus of H. taeniaeformis exhibited no signs of dimorphism and seemed of uniform

size. Even though Rothman (1963) reported the absence of microtriches on the scolex of H. diminuta, this observation has been refuted by Ubelaker et al. (1973) and Berger et al. (1971). Certain basic features found on the scolex such as ridges, depressions, folds and microtriches may assist the scolex in adhering to the mucosal wall of the host. Moreover, they increase its absorptive surface area and are thereby involved in the worms' uptake of nutrient materials and metabolism.

Microtriches were also found on the tegument of the strobila and bladder of the cysticercus of H. taeniaeformis. The tegument of the strobila region of this form presented no unusual structures such as those described as 'tumuli' by Boyce (1976) which were observed on the surface of the pseudophyllidean cestode, E. salvelini. The distribution of microtriches over the surface of the tegument of the bladder is in accord with the reports of Baron (1968) on Cysticercus longicollis and Nieland and Weinbach (1968) on C. fasciolaris with transmission electron microscopic studies. Their presence here suggests that the bladder is physiologically active and plays and important role in the uptake of nutrients. The existence of pores on the surface of the bladder is in accord with the observations of Rees (1951) and these may also possibly connect with the canals of the excretory system. Nieland and Weinbach (1968) reported similar ducts in TEM studies of the bladder and suggested metabolic

activity for those structures because of large accumulations of glycogen and numerous inclusions. Several other investigators have also suggested that the bladder of related forms is a specialized structure which plays an important role in nutrition and metabolism as well as a storage reservoir for growing strobila rather than functioning exclusively as an excretory structure (Šlais, 1966; Taylor et al. 1966).

Examination of the eggs of H. taeniaeformis by SEM at low and high magnifications revealed certain interesting surface features. At low magnifications, the surface of the eggs appeared to be smooth. However, at higher magnifications, the surface of the eggs was not smooth and consisted of irregular scale-like structures with denticles and bosses. Eggs believed to be in an earlier stage of development exhibited pit-like depressions and small spherical bosses distributed irregularly over the surface. Eggs treated with high concentrations of EDTA showed a distorted surface. When eggs were subjected to a low concentration of EDTA, no distortion was observed. Upon fractionation, the inner surface membrane showed irregular ridges and depressions. These observations were in accord generally with those of (Race et al. 1971; Ubelaker and Allison, 1972; Specian et al. 1973; and Ubelaker et al. 1976). The present study shows that the eggs of the cestode, H. taeniaeformis as revealed by SEM are similar to data obtained by other investigators on the eggs of trematodes, cestodes and nematodes.

According to Ubelaker et al. (1976) the presence of structural modifications such as opercula may represent a region permitting larval emergence. It is also thought by these investigators that pores in the opercular structure may penetrate into the chitinous layer and could possibly function as important avenues for the entrance of substances from the host which trigger hatching and may provide a weakened polar area of the egg shell for emergence of the larvae. In the current study of the eggs of H. taeniaeformis, the presence of bosses (spherical, knob-like structures on the surface of the egg) may represent an operculated structure that may facilitate larva emergence.

According to Ubelaker et al. (1976) the nature of factors responsible for the determination of the architectural pattern of the egg shell is unknown. It was indicated that the deposition of the materials released by the oocyte produces a species-specific pattern. Another possibility was that the eventual pattern was determined by the interaction of the developing shell with materials present in the uterine fluids. It was also mentioned that the differences in the pattern of sculpturing of the shells of certain species could be due to intrinsic and genetic peculiarities of the egg. On the basis of the results obtained using SEM techniques on the larvae and eggs of H. taeniaeformis, microscopical examinations of soft tissues can provide detailed information regarding complex three-dimensional relationships of surface structures.

Ultracytochemical studies on the cysticercus of H. taeniaeformis revealed alkaline phosphatase activity in the strobila region of the tegument. The reaction product was localized more intensely within the microtriches of the distal cytoplasm. Activity was also localized within the cytoplasm, processes of perinuclear cells, circular and longitudinal muscle bundles and in calcareous corpuscles. Several investigators have demonstrated alkaline phosphatase activity by histochemical methods for light microscopy (Rogers, 1947; Yamao, 1952a,b; Erasmus, 1957a,b; Kilejian et al., 1961; Bogitsh, 1963, 1967; Waitz, 1963; Waitz and Schardein, 1964 and Lee and Tatchell, 1964). These investigators were in agreement generally, that alkaline phosphatase activity was present in the cuticle, subcuticular cells and faintly around the muscles in the perinuclear region. The findings in our study are in accord with and substantiate light microscopic studies of all except Yamao (1952a) who reported that alkaline phosphatase activity was absent in the cuticular layers of adult cestodes, A. perfoliata, A. magna, M. benedeni, M. expansa and T. taeniaeformis but present in the basal membrane and the subcuticular cells. Yamao (1952b) found acid phosphatase alone in the larval cestode, C. bovis and alkaline phosphatase in E. cysticus fertilis. Alkaline phosphatase was also present in the epithelium of the excretory ducts of both species. The distribution of the two phosphatases in the body tegumental layer of C. fasciolaris

was identical to that of the adult cestode. Neither phosphatase could be detected in the epithelium of the excretory ducts of the larvae or adult of T. taeniaeformis.

The present study shows that in the cysticercus of H. taeniaeformis, alkaline phosphatase activity was present in considerable amounts within the microtriches of the distal cytoplasm. Activity was not found in the basal membrane of the distal cytoplasmic region and in the excretory ducts of the perinuclear cytoplasmic region. However, the demonstration of alkaline phosphatase activity in the perinuclear cytoplasmic region agrees with previous investigations which histochemical methods for light microscopy were employed. Erasmus (1957a,b) was unable to demonstrate alkaline phosphatase activity in the cysticercus of T. pisiformis and in Cysticercus tenuicollis using histochemical methods but did detect the enzyme by biochemical methods.

The few ultrastructural studies on alkaline phosphatase localization have been limited primarily to the microthrix brush border. Previous studies have not demonstrated alkaline phosphatase activity in the perinuclear cytoplasmic region. Rothman (1966), using histochemical methods for electron microscopy, demonstrated alkaline phosphatase activity on the outer membrane of the proximal microtriches in H. citelli. The precipitate accumulated on the surface of the proximal microtriches in such intensity that the intermicrotrichial spaces were obliterated. The activity in the cuticle proper and in the cuticular mitochondria was questionable. The alkaline

phosphatase controls showed a precipitate in the absence of the substrate. Our studies are somewhat in accord with those of the latter investigator. The reaction product was so intense within the microtriches that the intermicrotrichial spaces could barely be seen. However, no activity was seen in the mitochondria of the distal cytoplasm. Neither did control tissues incubated in a substrate-free medium reveal alkaline phosphatase activity. Lumsden et al. (1968) suggested that the lead precipitate in control tissues observed by Rothman (1966) was due to a high lead concentration of 3 mM and that a reduction to that of 2 mM would eliminate this precipitate artifact. In the present study no lead precipitate artifacts were observed in control tissues and this suggested that the concentration of lead employed was adequate.

In the ultrastructural preparations of the cysticercus of H. taeniaeformis, the enzyme activity appeared to be localized largely at the surface of the plasmalemma bordering the free surface of the strobila integument. Since the reaction product was inhibited by 1 mM of NaF and 10 mM of EDTA, the enzyme involved was considered to be alkaline phosphatase. These findings are in accord with the observations of Lumsden et al. (1968) in H. diminuta.

Dike and Read (1971) demonstrated two phosphatases biochemically and ultrastructurally in H. diminuta, using fructose phosphate and paranitrophenyl phosphate as substrates. The

differences in susceptibility of the hydrolysis of the substrates to pH and to various inhibitors provided evidence for at least two phosphohydrolases in the tegument. The findings in our studies were similar to those of Dike and Read (1971) in that the reaction product was strongly localized on the microthrix brush border. The number and specific characteristics of phosphohydrolases involved at the brush border in the cysticercus of H. taeniaeformis must await further study utilizing different substrates at varying pH levels.

The localization of alkaline phosphatase activity in calcareous corpuscles has not been reported by electron microscopic methods. In the present study the observations of alkaline phosphatase activity in calcareous corpuscles were in accord with those of Chowdhury and Ray (1962) in which histochemical methods for light microscopy were employed. These observations further confirm the findings of investigators who used biochemical analysis to demonstrate alkaline phosphatase activity in calcareous corpuscles (Scott et al., 1962; von Brand et al., 1960, von Brand and Pugh, 1965; Nieland and von Brand, 1969 and Smyth, 1969).

The function of alkaline phosphatase in helminths has been considered by many authors (Rogers, 1947; Bullock, 1949, 1958; Dusanic, 1959; Yamao, 1952a,b; Erasmus, 1957a,b; Phifer, 1960a, 1960b, 1960c; Kilejian et al., 1961; Bogitsh, 1963; Lee and Tatchell, 1964; Lumsden et al., 1968, Lumsden, 1975;

Dike and Read, 1971). These considerations usually involved digestion, absorption and secretion, elimination of fatty acid waste products, release of phosphate ions, mediated nutrient transport, carbohydrate metabolism, calcification and energy metabolism.

On the basis of the results obtained in this study, the alkaline phosphatase activity within the microtriches of the distal cytoplasmic region could suggest that the enzyme is involved in nutritional absorption and in the release of energy for metabolic processes at the tegumentary surface. The localization of the enzyme in processes of cells in the perinuclear region and around muscles may further be an indication that the phosphatases are playing a role in the transport of nutrients from the distal cytoplasmic surface to the perinuclear cytoplasmic region, thereby causing the release of energy and the utilization of nutrients by the cells. Since cytoplasmic localization of alkaline phosphatase was apparent in the perinuclear region, this may suggest involvement of the enzyme in intracellular metabolism as well as in transcellular transport.

The nervous system of cestodes has been studied extensively in whole specimens, homogenates and sectioned materials. Those studies have been conducted with histochemical methods for light microscopy in localizing specific and nonspecific cholinesterase activity. Little attention has been given to the fine structural localization of these enzymes. According to Bullock

and Horridge (1965) the reasons for this limitation are the lack of delimiting capsules around ganglia and cords and the lack of distinctive textual characteristics to separate nervous tissue from parenchymal components. The nervous system of tapeworms, according to Warble and McLeod (1952) is one of the most difficult anatomical features to study.

In the present study, acetylcholinesterase activity was localized in the scolex of the cysticercus of H. taeniaeformis. The reaction product in the form of electron dense deposits was localized within the microtriches of the rostellar region, excretory collecting ducts, flame cells, nerve cells and lateral nerve trunks. The presence of acetylcholinesterase activity within the microtriches is in accord generally with the findings of Rothman (1966), who demonstrated cholinesterase activity predominately on the distal microtriches of H. citelli and suggested that the enzyme was involved in the transport of materials. However, in the cysticercus of H. taeniaeformis, acetylcholinesterase activity was localized within proximal and distal portions of microtriches.

Schardein and Waitz (1965) found that esterases were plentiful in the cuticle and nervous system of H. diminuta, H. nana and H. taeniaeformis. H. taeniaeformis was the most reactive to methods of esterase and cholinesterase activity. With indoxyl acetate, alpha naphthyl and acetylthiocholine substrates, there was complete inhibition of the reaction following the pretreatment with 2×10^{-4} M

eserine. It was concluded that the enzyme hydrolyzing the acetate substrates at pH 7 was a specific cholinesterase; simple esterases would be inhibited by 2×10^{-4} M eserine but not 10^{-5} M. However, specific cholinesterase activity was sensitive to both concentrations. Chessick (1954) found that cholinesterases that are specific can hydrolyze acetates in addition to choline esters. In the cysticercus of the present study, acetylcholinesterase activity was inhibited by 10^{-4} M eserine. Activity in the rostellar region suggested that there are nerves innervating muscularized and physiologically active structures. Thus, acetylcholinesterase activity in the muscles of the rostellar region, flame cells and hooks may further indicate that nervous innervations associated with these structures are involved in some way with metabolic processes. Hence, enzyme activity associated specifically with nerve cells and other nerve components could suggest that the enzyme is playing a role in neurotransmission.

Lee et al. (1963) demonstrated nonspecific esterase and specific cholinesterase activity in the tissues of H. diminuta, H. citelli, H. microstoma and H. taeniaeformis. Specific cholinesterase activity was found in the cuticle of all cestodes studied and it was suggested that this specific enzyme may function in the transport of sodium. Lee and Tatchell (1964) described a nonspecific esterase in parts of the cuticle, subcuticle and nervous system of A. perfoliata. Various structures

were inhibited by 10^{-4} M eserine, suggesting that some of the esterase activity observed may be due to the presence of specific cholinesterase activity. These findings are in accord with those of Lee et al. (1963) who found specific cholinesterase activity in the nervous system and in the cuticle and subcuticular cells. Hart (1967) demonstrated specific cholinesterase activity in Mesocestoides corti in whole preparations. The entire scolex region revealed a black cap appearance. In the present study the cholinesterase activity was intense in regions of the rostellum of the cysticercus of H. taeniaeformis. These findings are also in accord with those of LeFlore and Smith (1976) who demonstrated specific and nonspecific cholinesterase activity in C. fasciolaris in whole preparations. All reactions for specific cholinesterase activity were abolished with 10^{-4} M or 10^{-3} M eserine. However, tissues incubated in a medium for nonspecific esterase activity were not affected by this concentration of eserine.

The findings in our study are also in partial agreement with those of Eränkö et al. (1968) who demonstrated specific and nonspecific cholinesterase activity in H. taeniaeformis by histochemical methods for light microscopy. The reactions using the substrates acetylthiocholine and butyrylthiocholine, with or without inhibitors showed identical distributions. Enzyme activity was detected in nerve trunks, nerve fibers and in the suckers. Since reactions were inhibited by 10^{-5} M

eserine, there was little doubt that the reactions were due to cholinesterases, even if these could not be divided into acetylcholinesterase and nonspecific cholinesterase. These workers suggested that cholinesterase activity in H. taeniaeformis was neuronal.

Teräväinen (1969b), using methods for electron microscopy, found that the distribution of acetylcholinesterase and nonspecific cholinesterase activity in the earthworm had identical distributions. Acetylcholinesterase activity was found within the nuclear envelope, nerve cells and peculiar lamellated bodies, as well as in reticulin fibers. Activity was inhibited by 10^{-5} M eserine. Our findings are in agreement with those of Teräväinen (1969b) except that acetylcholinesterase activity was inhibited by 10^{-4} M eserine. The observations reported in the present study are also in accord with other ultrastructural studies on acetylcholinesterase localization in various mammalian tissues where activity was present in the nuclear envelope, vesicles, fibers, cytoplasm, nerve cells and muscles (Teräväinen, 1969a; 1969c; Eränkö et al., 1967; Shimuzu et al., 1966). Shield (1969) demonstrated specific acetylcholinesterase activity in D. caninum and H. taeniaeformis and on the basis of inhibitors employed found no nonspecific cholinesterase activity. The observations in our study are in accord with those of Shield (1969) since only acetylcholinesterase activity was revealed.

In the present study, acetylcholinesterase activity in

the rostellar region of the cysticercus of H. taeniaeformis was in accord with studies made by Wilson (1965) who demonstrated acetylcholinesterase activity in the scolex of H. diminuta and H. nana. He suggested that in H. diminuta, extensive innervation of nerves in the rostellar region was primarily sensory in function, rather than a remnant of a muscular organ of attachment.

Observations in the present study of acetylcholinesterase activity in the cytoplasm and muscles of the rostellar region are in accord with those of Wilson and Schiller (1969) who demonstrated specific acetylcholinesterase activity in whole preparations of H. diminuta and H. nana. He indicated that in H. nana the nerves innervating the rostellar region were involved in neuromuscular regulation of rostellar organs, and in H. diminuta the apical organ may function as sensory chemoreceptors in the process of strobilation and apolysis. The function of acetylcholinesterase activity in nerve trunks and muscularized organs was presumed to be mainly neuromuscular. In the present study it is probable that the innervation of nerves in the rostellum as revealed by acetylcholinesterase activity may be evidence of neuromuscular transmission and the regulation of other activities of the rostellum.

Smyth (1969) found that many species of cestodes contain gland cells located in the scolex region which may secrete neurohormones that regulate growth of the strobila. Morseth (1967b) demonstrated ultrastructurally neurosecretory and

sensory processes in the larva and adult of E. granulosus. Davey and Brenckenridge (1967), using the techniques of Cameron and Steel (1959), described a group of nerve cells located in the rostellum which were said to undergo a secretory cycle associated with the development of the adult worm. They suggested that these cells could be responsible for regulating the process of strobilization. In the present investigation, nerve cells containing acetylcholinesterase activity were found in the rostellar region of the cysticercus of H. taeniaeformis. The heavy deposition of the reaction product within the nuclear envelope and cytoplasm of these cells may be an indication of the production and transport of acetylcholinesterase in cholinergic nerve cells.

The observed reaction product localized in flame cells of the cysticercus of H. taeniaeformis in this study was inhibited by 10^{-4} M eserine. This was, therefore, considered as evidence of acetylcholinesterase activity in these structures. According to Smyth (1969) the fat in cestode tissue is a by-product of carbohydrate metabolism and is voided by means of excretory canals. This investigator suggested that esterases associated with the walls of excretorty canals in species H. diminuta and H. taeniaeformis were engaged in the secretion of lipids. Arme (1966) suggested that esterases associated with similar structures in L. intestinalis may be an indication that the enzymes are playing a role in the excretion of lipids which accumulate as metabolic by-products.

Schwabe et al. (1961) demonstrated specific acetylcholinesterase activity in homogenates of E. granulosus and suggested that the enzyme was involved in permeability control and osmoregulation. Acetylcholinesterase activity in the flame cells of the cysticercus of H. taeniaeformis may serve the same function since the excretory system is one of osmoregulation and is responsible for the elimination of waste products.

On the basis of the observations found in this study, acetylcholinesterase activity in the cysticercus of H. taeniaeformis may be involved in neuromuscular and sensory transmission, transport of nutrients across the tegument, and secretion and excretion of metabolic substances.

CHAPTER VI

SUMMARY

1. SEM of the scolex of the cysticercus of H. taeniaeformis revealed certain distinct surface features:
   (a) The suckers of the scolex were composed of large surface folds, depressions and ridges. At high magnifications microtriches were observed within the cavities and over the rims of suckers.
   (b) The rostellum consisted of a double crown of hooks which projected from large oval-shaped cavities.
   (c) Indentations and folds were observed on the scolex proper.
2. The tegument of the strobila region was studded with bosses of uniform size which were revealed as microtriches at higher magnifications.
3. The bladder consisted of densely packed microtriches and lateral and terminal excretory pores.
4. SEM of the eggs of H. taeniaeformis revealed distinctive scale-like patterns, small, spherical bosses and pit-like depressions. The membrane of fractured eggs consisted of a thick inner surface of ridges. The surface of eggs subjected to a high concentration of EDTA showed distortion and the emerging hooks of the embryo.
5. The substrate, b-glycerophosphate, was used to demonstrate the ultrastructural localization of alkaline phosphatase activity. Alkaline phosphatase activity was inhibited by

1 mM NaF or 10 mM EDTA. The reaction product was localized within the microtriches of the distal cytoplasm, cytoplasm and processes of perinuclear cells, circular and longitudinal muscle bundles and in calcareous corpuscles.

6. The substrate, acetylthiocholine iodide, was used to demonstrate the ultrastructural localization of acetylcholinesterase activity in the scolex. Acetylcholinesterase activity was inhibited by 10^{-4} M eserine. The reaction product was localized within the microtriches of the distal cytoplasm, muscle bundles, cytoplasm, fibrous tissue and vesiculated bodies of the rostellar region. Activity was also demonstrated within the nuclear membrane, cytoplasm and membrane of nerve cells, flame cells, and hooks of the rostellum.

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